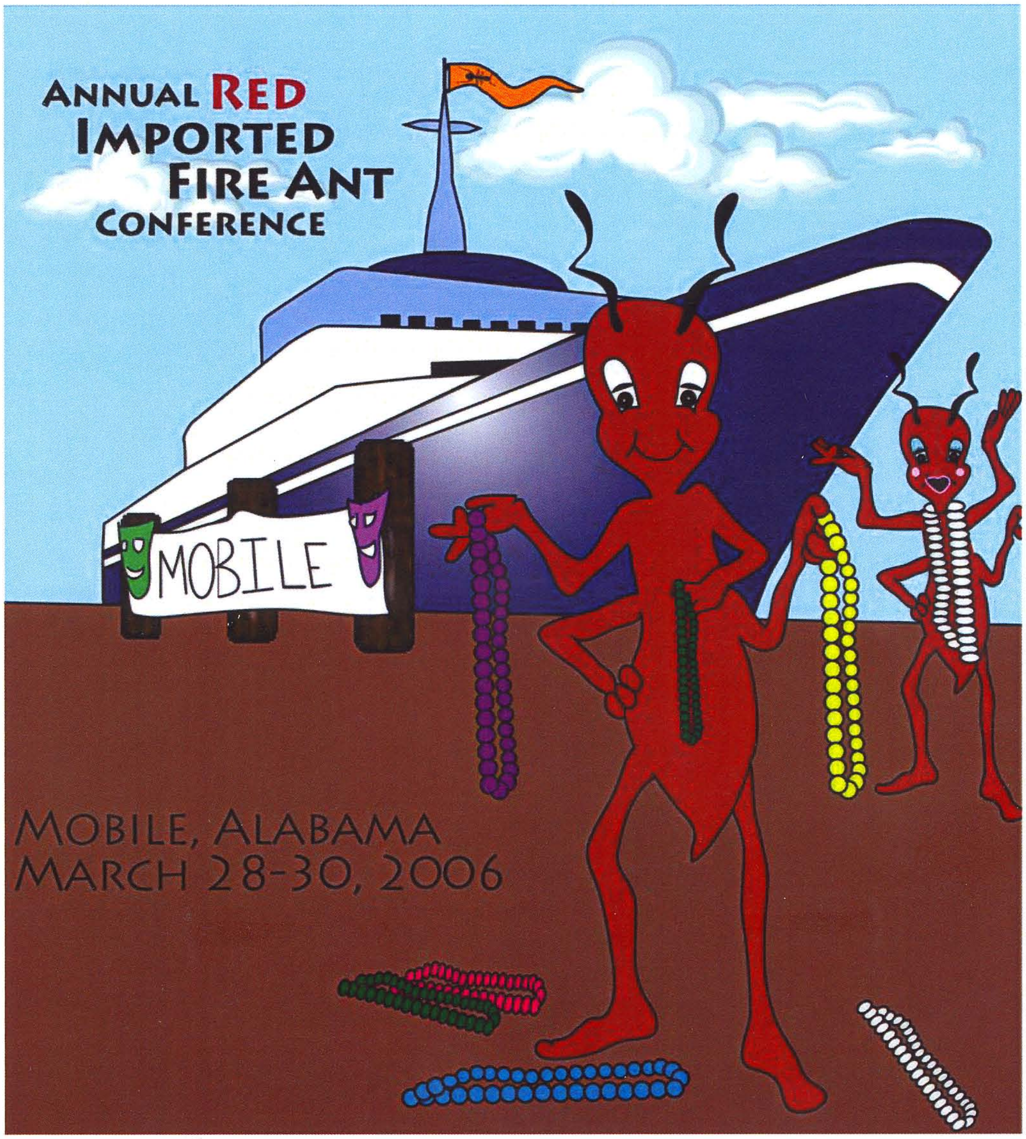

Conference Proceedings

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Auburn University | Entomology and Plant Pathology

Proceedings of the
Red Imported Fire Ant Conference
March 28-30, 2006
Mobile, Alabama

Arrangements Committee

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This publication is the result of a special Red Imported Fire Ant Conference organized by the Alabama Fire Ant Management Program at the Department of Entomology and Plant Pathology at Auburn University.

The opinions, conclusions, and recommendations are those of the participants and are advisory only. Trade and company names are used in this publication solely to provide specific information. Mention of a trade or company name does not constitute a warranty or an endorsement by the Alabama Fire Ant Management Program, the Department of Entomology and Plant Pathology, and/or Auburn University to the exclusions of other products or organizations not mentioned.

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The 2007 Imported Fire Ant Conference will be held in Gainesville, FL April 24 - 25, 2007. The 2008 Imported Fire Ant Conference will be hosted by Clemson University.

A copy of the Proceedings may be obtained from:

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Acknowledgements

The success of the 2006 Imported Fire Ant Conference was a result of the efforts of the planning committee, the Alabama Fire Ant Management Program, the Department of Entomology and Plant Pathology, our corporate sponsors and, of course, all who took time to prepare and present the information that can be used to manage imported fire ants for our clientele through safe, effective and affordable means. Without the excellent cooperation, participation and support of everyone, the conference would not have been possible.

Special thanks are due to many on the committee. Arthur Appel and Micky Eubanks were responsible for the coordination, scheduling, arrangement and publication of the meeting agenda. It looked great. Kathy Flanders and Leisha McDaniel were responsible for registration and receipts and kept everything under control as long as I stayed out of the way. Chazz Hesselein and Mike Williams helped with on-site registration. A very special thank you is reserved for Vicky Bertagnolli for her help. She collected presentations and organized them for projection, gathered registration gifts from local sponsors, organized the daily door prize drawings, collected and organized the articles for the proceedings, manned the projector for most of the conference and put up with me for the six months preceding the conference.

Thanks go to Carol Russell, USDA/APHIS/PPQ, Hawaii. Even though she was not able to attend the conference, she provided all of the goodies for the door prizes, especially the wonderful candy and coffee, and the leis. Thanks to Patty Beckley for delivering the leis and attempting to publicly embarrass me, a very difficult task.

Thank you to Alicia McCray, Valerie Jones, Richlyn Lynch, Stan Martin and the staff of the Radisson Admiral Semmes Hotel and Suites for their assistance and patience with the set up, catering, room accommodations, and last minute changes.

I want to thank Dr. David Williams, University of Florida, Gainesville for his keynote presentation, "A Brief History of the Red Imported Fire Ant in the US" and for allowing it to be placed on the web for use by others. I also want to express gratitude to Dr. David Oi, USDA, Gainesville for his entertaining surprise introduction of the keynote speaker.

Many thanks are due to all of the conference sponsors and supporters. Their support is essential in making the conference financially possible. Their ads can be found at the end of the proceedings. Thank you to the Alabama Peanut Producers, the Auburn University Bookstore, Cotton Counts and the Mobile Bay Convention and Visitors Bureau for registration gifts.

AGENDA

ALL MEETINGS IN LOCATION

TUESDAY, MARCH 28

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7:00 – 8:00 pm	**Poster Setup** – State Room B & C

WEDNESDAY, MARCH 29

7:00 am – 1:00 pm	**Registration**
7:00 – 8:00 am	**Poster Setup** – State Room B & C
7:15 – 8:00 am	**Continental Breakfast** – Mezzanine
8:00 am	**Call To Order** – Crystal Ballroom
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8:20 – 8:30 am	**L. C. "Fudd" Graham:** Special Presentation
8:30 – 9:30 am	**Keynote Address by Dr. David Williams, University of Florida**: A brief history of the red imported fire ant in the U.S

Session: Chemical Control

Moderator:	**Fudd Graham**
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9:50 – 10:05 am	**J. Altom:** Esteem ant bait now labeled for pasture and hay
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11:15 – 11:30 am	**D. Vander Hooven:** TAST-E-Bait, a new improved bait carrier
11:30 – 11:45 am	**T. Rashid,** P. Parkman, J. Oliver, K. Vail: Mortality response of red, black and hybrid imported fire ants to insecticide treated soil in laboratory bioassays

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3:30 – 3:45 pm	**S. Ochleng:** Imported fire ant repellency and mortality following exposure to Ecotroll EC
3:45 – 4:00 pm	**R. Renthal,** D. Velasquez, D. Gonzalea, A. Cassill, S. Ballji, G. Sunter: Chemical signaling and fire ant behavior
4:00 – 4:15 pm	**R. Vander Meer,** C. Preston: The effect of biogenic amines on fire ant conspecific aggression
4:15 – 4:30 pm	**A. Rao** and S. Vinson: Larval regulation of reproduction in the red imported fire ant
4:30 – 4:45 pm	**J. Chen**: Chemicals incorporated in nest material by red imported fire ants
4:45 – 5:00 pm	**M. Wang,** J. Oliver, D. Fare: Fire ant repellents from plant extracts
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8:30 – 8:45 am	**D. Oi,** J. Briano: Host specificity and transovarial transmission of *Vairimorpha invictae*
8:45 – 9:00 am	**S. Porter,** L. Calcaterra, L. Varone, J. Briano: New biocontrol agents from Argentina
9:00 – 9:15 am	**S. Valles:** *Solenopsis invicta* viruses 1 and 1A: Phenology, geographic distribution, and species specificity
9:15 – 9:30 am	**S. Vinson,** T. Azizi and K. Snowden: The historical occurrence of *Thelohania solenopsae* in red imported fire ant (*Solenopsis invicta*) colonies in the Brazos valley region of Texas.

Session: Biology & Ecology

9:30 – 9:50 am	**M. Eubanks,** L. Cooper, J. Murphy: Fire ants can increase the spread of aphid-vectored plant viruses
9:50 – 10:05 am	**D. Henne,** S. Johnson: Characteristics of *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) population spread in Louisiana
10:05 – 10:35 am	30 Minute Break
Moderator:	**Vicky Bertagnolli**
10:35 – 10:55 am	**A. Lee,** C. Husseneder, L. Hooper-Bùi: Culture-independent identification of bacteria in fourth-instar red imported fire ant larvae, *Solenopsis invicta* Buren
10:55 – 11:10 am	**L. Luo:** The red imported fire ant, *Solenopsis invicta* in mainland China
11:10 – 11:30 am	**K. Rice,** M. Eubanks: Effect of fire ants on an old field arthropod community
11:30 – 11:45 am	**J. Vogt,** B. Wallet: Modeling thermal dynamics of imported fire ant mounds
11:45 am – 1:00 pm	Lunch (on your own – return by 1:00 pm)
Moderator:	**Chip East**
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1:15 – 1:35 pm	**L. Hooper-Bùi,** B. Wiltz: Effect of Hurricane Katrina flooding on Ants of New Orleans and St. Bernard parishes, Louisiana: Part II
1:35 – 1:55 pm	**L. Womack,** L. Hooper-Bùi, M. Chamberlain, B. Moser: Impacts of red imported fire ants (*Solenopsis invicta* Buren) on native faunal communities in two pine-dominated ecosystems
1:55 – 2:10 pm	**C. Yang,** C. Shih, W. Wu: Molecular evidence on the origin of red imported fire ant *Solenopsis invicta* in Taiwan

Session: Eradication Efforts

2:10 – 2:25 pm **C. Cassidy,** G. Goedhart, J. Green, M. Hearst, L. Shaw: Residential R.I.F.A. treatments in Orange County, CA

2:25 – 2:55 pm 30 Minute Break

Moderator: **Willie Datcher**

2:55 – 3:10 pm **C. McNicol:** Surveillance methodologies used within Australia. Various methods including visual surveillance and extraordinary detections; above ground and in ground lures

Session: Extension / Public Education

3:10 – 3:25 pm **K. Flanders,** B. Drees: eXtension: Taking the sting out of imported fire ants

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3:25 – 3:40 pm **R. Wright,** W. Smith, V. Karpakakunjaram: Update on the area-wide fire ant management project in Oklahoma

3:40 – 3:55 pm **J. Haffenden:** Movement controls used to control red imported fire ant in Australia. Various mechanisms utilized including steam machine, crushers, flame thrower machine and mulching processes

3:55 – 4:10 pm **C. Brown** and A. Callcott: A Regulatory Perspective on Fire Ant Research

4:10 – 4:25 pm **A. Bhatkar:** The edging fire ant and the buzzing bees

4:25 – 4:40 pm **K. Loftin,** J. Hopkins: IFA quarantine treatment for commercial sod: Arkansas experiences

4:40 – 4:55 pm **C. Russell:** Pacific Invasive Ant Conference: Hawaii 2007

4:55 – 5:10 pm **Wrap Up** – Next Meeting Site

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2. Acoustic and high-speed videographic analysis of flying imported fire ants. **L. Gui,** C. Talmadge, R. Hasse, T. Fink, Z. Cao, Y. Wang, J. Seiner, D. Streett, A. Pranschke

3. Stridulation behavior in *Solenopsis invicta* and *S. richteri*. **J. Marquess,** J. Anderson

Session: Biological Control

4. Arkansas' first *Pseudacteon curvatus* release. **J. Clemons,** A. Simpson, J. Hopkins, K. Loftin

5. Distribution patterns of *Thelohania solenopsae* in the RIFA populations of southeastern Oklahoma: Implications. **V. Karpakakunjaram,** A. Chan, R. Wright

6. Expansion of *Pseudacteon tricuspis* in Arkansas. **M. McCarter,** D. Petty, G. Gavin, R. Herring, J. Clemons, R. Dollar, J. Hopkins, K. Loftin

7. Host location behavior of *Pseudacteon curvatus* (Diptera: Phoridae) in mono- and polygyne fire ant colonies (Hymenoptera: Formicidae). **V. Bertagnolli,** F. Graham

8. *Pseudacteon* spp. (Diptera: Phoridae) range expansion in Alabama. **F. Graham,** V. Bertagnolli, K. Ward, R. Ward

9. The effects of food supplement on lifespan and nutrient metabolism in the parasitic phorid fly, *Pseudacteon tricuspis*. **L. Chen,** H. Fadamiro

10. Update on USDA, APHIS phorid fly releases and monitoring efforts. **R. Weeks,** A. Callcott

11. Compensatory foraging strategy of red imported fire ant (*Solenopsis invicta*) colonies after exposure to introduced dipteran parasitoids (*Pseudacteon tricuspis*). **R. Puckett**, M. Harris, C. Barr

Session: Biology & Ecology

12. Cloning, sequencing, and expression of arginine kinase gene from the fire ant, *Solenopsis invicta* (B.). **N. Liu,** H. Wang

13. Diel and seasonal phenology and spatial dynamics of *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) in Louisiana. **D. Henne,** S. Johnson

14. Making wax casts of imported fire ant mounds. **D. Petty,** K. Loftin, J. Hopkins

15. Phylogeography of the fire ant *Solenopsis invicta*. **M. Ahrens,** D. Shoemaker

16. Survey of red imported fire ant populations in north central Mississippi. **D. Streett,** M. Allen, A. Pranschke

17. The distribution of imported fire ants (Formicidae: *Solenopsis* spp.) in Christmas tree plantations across Mississippi. **S. Self**

18. The distribution of imported fire ants (*Solenopsis* spp.) and their potential foraging competitors in relation to local conditions and interspecific competition in Mississippi forests. **T. Menzel,** T. Nebeker

19. Identification of the gene(s) involved in the early events concerning flight muscle degeneration in the Red Imported Fire Ant (*Solenopsis invicta*). **T. Azizi,** S. B. Vinson

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20. Entomological, economical and philosophical considerations concerning automated detection and treatment of fire ant colonies. **J. Reed,** F. To, D. Smith

21. Evaluation of BAS 3201 bait and drench applications for the control of imported fire ants. **W. Hudson,** S. Diffie

22. Evaluation of homeowner formulations of indoxacarb, hydramethylnon, pyriproxyfen and fipronil against imported fire ants. **J. Hopkins,** D. Petty, K. Loftin

23 Evaluation of hydramethylnon bait and various contact insecticides as individual mound treatments against imported fire ants. **J. Gavin,** K. Loftin, J. Hopkins, D. Shanklin

24. Fire ant management at poultry houses. **D. Pollet**

25. Using population index and foraging activity to evaluate broadcast applications of baits and granular insecticides for imported fire ants. **T. Rashid,** P. Parkman, J. Oliver, K. Vail

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26. Bifenthrin and chlorpyrifos in reduced radius band treatments for in-field application on nursery plants. **S. James,** L. McAnally, A. Callcott

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27. Area-wide suppression of fire ants: demonstration project in Mississippi, 2005. **D. Streett,** A. Pranschke, J. Vogt, J. Reed, A. Callcott

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28. The pest ants of Louisiana: a guide to their identification, biology and control. **S. Dash,** L. Hooper-Bùi, M. Seymour, L. Womack, B. Corns

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Fire Ants at Talladega
Bringing NASCAR Fans Back Down to Earth

Henry D. Dorough
Regional Extension Agent
Animal Science & Forages
Alabama Cooperative Extension System
Talladega, Alabama

Since their introduction into the United States in the early 1900's, fire ants have been the primary target of many Extension projects. One recent project involved treating for fire ants at the Talladega Superspeedway, NASCAR's fastest track and home of two major NASCAR Nextel Cup races in Talladega, Alabama. Twice each year about 150,000 race fans converge on campgrounds owned by the Talladega Superspeedway. An additional 100,000 fans will arrive on race day and picnic in the day parking areas. Track officials and race fans routinely called the local Extension office to get recommendations for controlling fire ants in the campgrounds at the track. Twice a year, race fans poured into local stores to buy anything that purported to kill fire ants, even resorting at times to illegal methods such as the use of gasoline, diesel fuel and lighter fluid within infested areas.

A visit with track officials revealed that fire ants were the number one complaint from race fans camping on speedway property. Track officials had been giving zip-lock bags of Arndro to campers that complained of fire ant problems; a practice that has obvious flaws. Amdro requires two to four weeks to control fire ant populations whereas race fans are only at the track for a maximum of one week for each race. Also, without specific label instructions, intoxicated race fans are hardly likely to apply the bait according to label directions.

After visiting with track officials, a team of Extension personnel along with the Alabama Fire Ant Management Program worked together to develop the largest site-specific demonstration ever conducted in the State of Alabama.

The primary project goals for the team were:

- To educate Talladega Superspeedway employees on proper bait calibration and application techniques.
- To educate race fans on effective fire ant control methods.
- To minimize the environmental impact of race fans killing fire ants on speedway property.

The goals for Talladega Superspeedway officials were:

- To eliminate fire ant complaints from campers in the more expensive campgrounds.
- To eliminate fire ants during race week.

Pre-treatment surveys of speedway property revealed an average fire ant population of 186 mounds per acre. The infield at the track had a population of 243 mounds per acre. From August 1 through 3, 2005, the team equipped 16 John Deere Gators with Herd GT-77 seeders, trained the speedway staff in calibration and spreading techniques and then monitored the spreading of over 2,500 pounds of Extinguish Plus on approximately 1,500 acres of campgrounds owned by the Talladega Superspeedway.

On August 31, 2005, 30 days after the initial treatment, the population was reduced to just 49 mounds per acre. Speedway officials, although impressed with the results, insisted on a zero tolerance for fire ants during race week in October. Therefore, the decision was made to make an additional application of Extinguish Plus to the most expensive campgrounds inside and outside of the track where the greatest fire ant populations existed and where the most camper complaints originate. On September 23, 2005, nine days before the Nextel Cup championship points race, the population was reduced to only nine (9) mounds per acre, an overall 95 percent reduction. The most expensive campgrounds in the infield were practically devoid of fire ants. During race week, the team surveyed approximately 10 percent of the campers about their experiences with fire ants at Talladega. Fans were asked to rate past and present experiences on a scale of 1 – 10, where 1 was no problem and 10 was an extreme problem. First-time campers rated their present experience at 1.3. Fans camping at Talladega two to four years rated their past experience at 4.1 and their present experience at 1.2. Fans camping five or more years rated their past experience at 6.9 and their present experience at 1.4. The numbers clearly indicate that race fans considered fire ants a serious issue at their campsites in past years and that the treatment with Extinguish Plus greatly improved their overall camping experience.

Fans were asked if they had brought chemicals to control fire ants in their campsite and if so, if they were used since arriving on speedway property. Thirty-nine percent indicated they brought chemicals with them and only 47 percent of those individuals said they actually used the product. In all, 80 percent of the campers used no chemicals to control fire ants in their campsite. Of those who used the chemicals they brought, most said based on past experiences they were in the habit of treating their campsite prior to parking their RV and did so before learning of our project. The list of products brought with the campers included a variety of contact insecticides, baits, aerosols as well as gasoline, diesel fuel and lighter fluid.

Fans were also asked if they had fire ants where they lived and if so, how they controlled fire ants within and outside their homes. Sixty-seven percent said they lived in a fire ant infested area. The rest resided outside areas of the U.S. where fire ants have invaded. For those with fire ants at home, most said the same methods used at the Talladega Superspeedway were used at home while some indicated they used pest control operators to treat their personal property. When asked to rate the effectiveness of the treatments they used, the results were the same for treatments on speedway property and at home. Using the same scale of 1 – 10, fans rated their control methods an average of 5.7. Contact insecticides rated 5.2 and baits rated 6.2.

On an interesting note, the fans surveyed represented 24 states including: Alabama, Arizona, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kentucky, Louisiana, Maine, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Jersey, New York, North Carolina, Ohio, South Carolina, Tennessee, Texas and Virginia.

In addition to the surveys, race fans were educated about the method of fire ant control used at Talladega. Literature was handed out giving details of the Talladega project, results and information on controlling fire ants at home with a bait product using the two-step method. Several local newspapers assisted with the educational effort by publishing articles on the project's results. Signs also were posted throughout the speedway property to inform fans about the project.

Seven months after the initial treatment, control of fire ants at the speedway remained at 95 percent. During the spring race in April 2006 there were no complaints registered with track officials by campers on speedway property. A one year survey of fire ant populations at the Talladega Superspeedway is planned and the results will be added to this report at that time.

In summary, the project was a tremendous success. All of the project goals were met. Talladega Superspeedway employees learned the proper methods for controlling fire ants using a bait product, race fans were educated about fire ants and their control and Talladega Superspeedway property was protected from the tremendous volume of chemicals normally used to control fire ants during race week twice each year. The project was highly successful not only for its effect in controlling fire ants but through its use of environmentally sound and sustainable methods. It is a success not only reflected by the survey but by the positive comments of race fans who, by becoming better informed about effective fire ant control methods, were less inclined to resort to harsh and, in many cases, largely ineffective control measures.

Esteem® Ant Bait Now Labeled For Pasture and Hay

John V. Altom, Field Market Development Specialist

Valent USA Corporation, 3700 NW 91st Street, Building C Suite 300, Gainesville, FL 32606

The active ingredient in Esteem® Ant Bait is pyriproxyfen. This is the same active ingredient (ai) that is in Knack® and Esteem® and used as foliar sprays to control insects such as whiteflies and scales. The pyriproxyfen is dissolved in soybean oil and applied to corn grit granules at 0.5% ai by weight. Pyriproxyfen is an insect growth regulator that is similar to naturally occurring insect growth hormones which interferes with (stops) the insect's growth and development.

Worker ants are attracted to the bait and carry the bait granules back to the ant colony. The worker ants ingest the soybean oil and ai and feed this to the queen and immature ants. The queen stops laying viable eggs and the immature ants are unable to development into adults. Consequently, worker ants are not replaced and the existing worker ants naturally die off. Within **3** to 4 weeks the ant colonies are declining and by 8 weeks very few ants can be found in the treated areas.

The best application method is via broadcast, either by ground equipment or aerial equipment. The rate is 1.5 to 2 lbs of formulated product per acre for pasture and hay. Ant species that are controlled with Esteem Ant Bait include the red imported fire ant, southern fire ant, pavement ant, and big-headed ant. Additions are currently being made to this label to include all imported fire ants.

The best time to apply Esteem® Ant Bait is when the worker ants are foraging. The most favorable air temperature range is between 65 and 85°F and when soil temperature is above 60°F. The presence of moisture (dew, rainfall, or irrigation) will reduce the effectiveness and attractiveness of the bait and should be avoided. Under extremely heavy fire ant populations, two applications will be required. One in late spring to early summer followed by another application in late summer to early fall.

Esteem® Ant Bait is currently labeled on over 100 crops (refer to label for specifics on each crop). With respect to the pasture and hay label, there are no grazing restrictions to livestock. The current package size is a 25-pound bag and a 2-pound bag will be introduced later in 2006. Valent USA will be evaluating the potential to blend Esteem® Ant Bait with various fertilizers throughout 2006 in an effort to save an application cost and provide growers with an easier application method through commercial fertilizer spreaders.

Mixing Bait with Fertilizer: Is It OK Yet?

Charles L. Barr, Extension Program Specialist
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One of the most common questions asked about broadcast fire ant baits is whether they can be mixed with fertilizer. There are a number of advantages to this practice. One is the simple cost savings of only needing to make one trip across the field. A second is eliminating the need for a second, specialized spreader for the bait. Probably the most important advantage, though least obvious, is that mixing bait with fertilizer eliminates the difficulty of calibrating a bait spreader to deliver the required low volume, usually 1.5 lbs/acre. Bait calibration changes with a host of factors: temperature, humidity, roughness of terrain, brand and lot of bait, to name a few.

Without calibrating before each application (even over the course of a day), it is easy to apply double or even triple the amount of bait, resulting in material shortages and greatly increased cost. Fertilizer spreader calibration, on the other hand, stays relatively constant under different environmental conditions. It is also much easier and more accurate to calibrate a spreader applying several hundred pounds per acre. In other words, a pound or two per acre difference in fertilizer application makes little difference in performance or cost, whereas the same error would double the cost of a bait application.

Unfortunately, tests conducted by the Texas Agricultural Extension Service in the 1990's indicated that pre-packaged bait mixed with fertilizer quickly lost its attractiveness to fire ants rendering it ineffective. Therefore, the practice was strongly discouraged. It was hypothesized that the fertilizer either absorbed the toxicant-containing soybean oil from the bait granules, making it unavailable to the ants, or rendered the oil unpalatable by transferring salts to it.

However, citrus growers in Florida reported that mixing Extinguish™ bait (0.5% s-methoprene) with fertilizer had become a regular part of their orchard maintenance program with excellent fire ant suppression. Wellmark International, makers of Extinguish, sponsored tests in 2003 and 2005 to try to directly address this contradiction. Another test using a different bait active ingredient was conducted in 2005 to help support results from the first two. Still another test, unintentional though it was, helped answer the question of whether oil absorption or unpalatability is the culprit in the performance failure of pre-mixed bait.

Pre-mixed bait + fertilizer blend performance failures

In 1994 three premium grade, encapsulated fertilizers were mixed with Amdro® (0.73% hydramethylnon) and applied through a tractor-mounted, PTO-powered fertilizer spreader. These mixtures were made at the factory and sat in the bags for some months. Another treatment used the same fertilizer spreader, but a Herd GT-77 seeder was mounted to it so the fertilizer and bait could be applied simultaneously, but without mixing. Amdro alone, applied with the Herd seeder was included as standard.

As shown in Figure 1, all three pre-mixes failed to show much control. The Amdro alone showed a typical performance curve while the simultaneous application actually performed both better and faster than the Amdro alone. The obvious conclusion was that pre-mixing fertilizer with bait cause the bait to become either ineffective or unpalatable to the ants resulting in little or no control.

That same year, a similar test was conducted using Logic/Award (1.0% fenoxycarb) as the bait mixed with another encapsulated fertilizer. As before, the mixture sat in the bags for some weeks before application. As shown in Figure 2, the pre-mixed blend showed little or no control compared to untreated plots. Though typically slow-acting, the Award standard reduced the number of active mounds over the 9-month course of the test.

Mix-and-apply blends of broadcast bait and fertilizer

As mentioned, Wellmark International sponsored a test in 2003 in which Extinguish was mixed with an agricultural grade fertilizer (20-5-10) to try to replicate the satisfactory fire ant control found by Florida citrus growers. The fertilizer was purchased the day before application and a thunderstorm hit the area that afternoon. The fertilizer, sitting in the back of a pickup truck, was packed in plastic bags. Nevertheless, some of the bags leaked and the fertilizer contents were wet by the morning of application. Consequently, the bait was exposed to not only the salts of the fertilizer, but also moisture, two factors that we felt sure would render the bait ineffective.

Nevertheless, as shown in Figure 3, the mix-and-apply Extinguish + fertilizer worked as well as Extinguish alone, though it took over 9 months to reach full mound suppression.

It was felt that these surprising results warranted another trial using equipment more like what would be used by agricultural producers. The treatments in the previous trial were applied with a Herd GT-77 electric seeder on quarter-acre plots. The next trial was applied using a tractor-mounted, PTO-powered fertilizer spreader on one acre plots. Treatments included both mix-and-apply and a treatment where Extinguish was mixed with the fertilizer, returned to the fertilizer bags and "aged" for two weeks prior to application.

Figure 4 shows the results of this field trial. As before, the mix-and-apply bland performed at fast and as well as Extinguish alone. Note that the pre-mixed material also reached a level of maximum suppression similar to that of Extinguish and the mix-and-apply blend. However, its activity was delayed almost two months versus the other treatments. We can offer no explanation for this phenomenon.

To add support for the feasibility of recommending the mix-and-apply treatment, another test was conducted in the fall of 2004 using a different bait, Advion® (0.045% indoxacarb), and a different fertilizer, 13-13-13. As shown in Figure 5, results were similar with the mix-and-apply blend working as fast and as well as Advion alone.

Absorption of oil or unpalatability?

The previous three tests provided strong data showing that the mix-and-apply method of applying bait and fertilizer was effective in controlling fire ants. However, the question of why the early tests showed poor control remained unexplained. Had the fertilizer granules absorbed the toxicant-carrying oil from the bait or had the salts in the fertilizer rendered the bait unpalatable to the ants?

An unexpected, potentially disastrous, event provided evidence that helped answer this question. In May 2004, we were in the process of applying a large test using different formulations of indoxacarb bait at the municipal airport in Palestine, Texas. We had completed applying treatments along both sides of the main runway when a boom-sprayer truck literally appeared over the horizon spraying our already-treated plots with fertilizer and, possibly, herbicide. It then sprayed a line of plots that we were about to treat.

At that point, we reasoned that the test was either ruined or the liquid fertilizer would have no effect, so we continued to apply treatments in the wetted vegetation. Eleven treated plots were oversprayed and five plots were sprayed prior to bait treatment. Fortunately, one untreated plot was also sprayed. Figure 6 shows that all plots performed as expected with no delay in speed of control and no reduction in maximum control.

It can be assumed that the liquid fertilizer contained similar, if not identical, chemicals as the granular fertilizer used in previous tests. With no chance of oil absorption onto fertilizer particles, had the fertilizer been repellent to ants or rendered the soybean oil unpalatable, the treatments should not have worked. However, they worked as well as the non-sprayed treatments. The success of the bait mixed with wet granular fertilizer in the 2003 test also adds supporting evidence.

Therefore, if the bait was not affected by the fertilizer ingredients without the particles, it can be reasonably assumed that it is the particles themselves that cause fire ant bait to "go bad" when they are mixed for a period of time. The likely culprit is that the fertilizer particles simply absorb the toxicant-carrying soybean oil from the corn grit making it unavailable to the ants.

Mixing Bait with Fertilizer: Is It OK Yet?

The answer to this question is an unequivocal, "Yes, no and maybe." Yes, if fire ant bait is mixed with fertilizer and applied immediately (within a few hours) it should work as well as the bait applied alone. No, if bait is mixed with fertilizer and allowed to sit for some time, on the order of weeks as would be found in a commercially produced blend, the soybean oil will likely be absorbed by the fertilizer rendering it ineffective. Maybe. The time between a few hours and a few weeks during which the components are allowed to stay in contact with each other is the gray area. At this point, mixing bait and fertilizer more than a few hours ahead of application should be avoided. Even allowing them to sit overnight should be considered questionable. It must be noted that at this time, no company endorses the mixing of its fire ant bait with fertilizer. Therefore, any mixing of products becomes the user's responsibility.

Mixing bait with fertilizer provides the advantages of ease and accuracy of calibration, no need for special bait spreading equipment and saving an entire pass across the area - all factors with great cost saving potential. Based on the data outlined above, mixing and immediately applying bait with fertilizer is a viable means of effectively controlling fire ants.

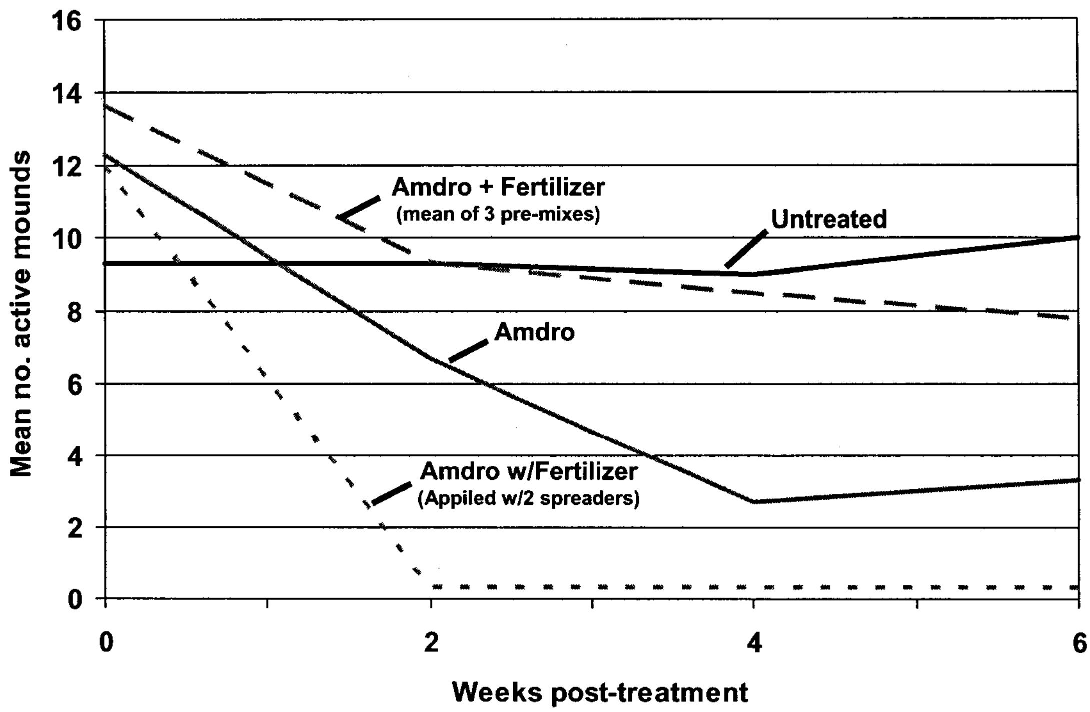


Figure 1. Amdro + fertilizer pre-mix, 1994

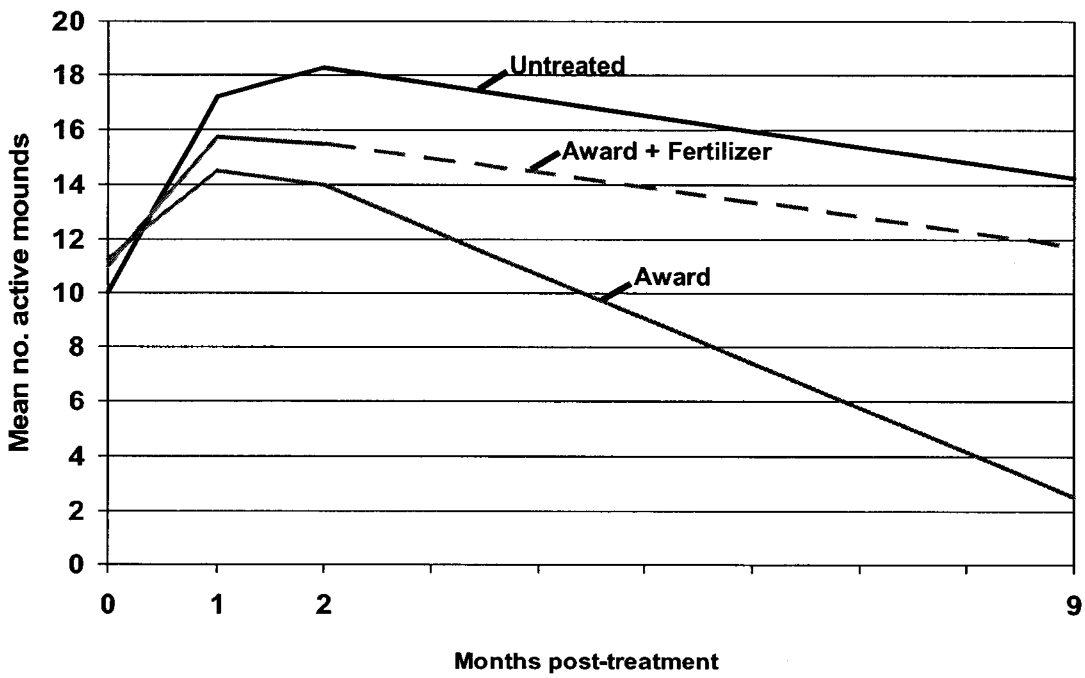


Figure 2. Award + fertilizer pre-mix, 1994

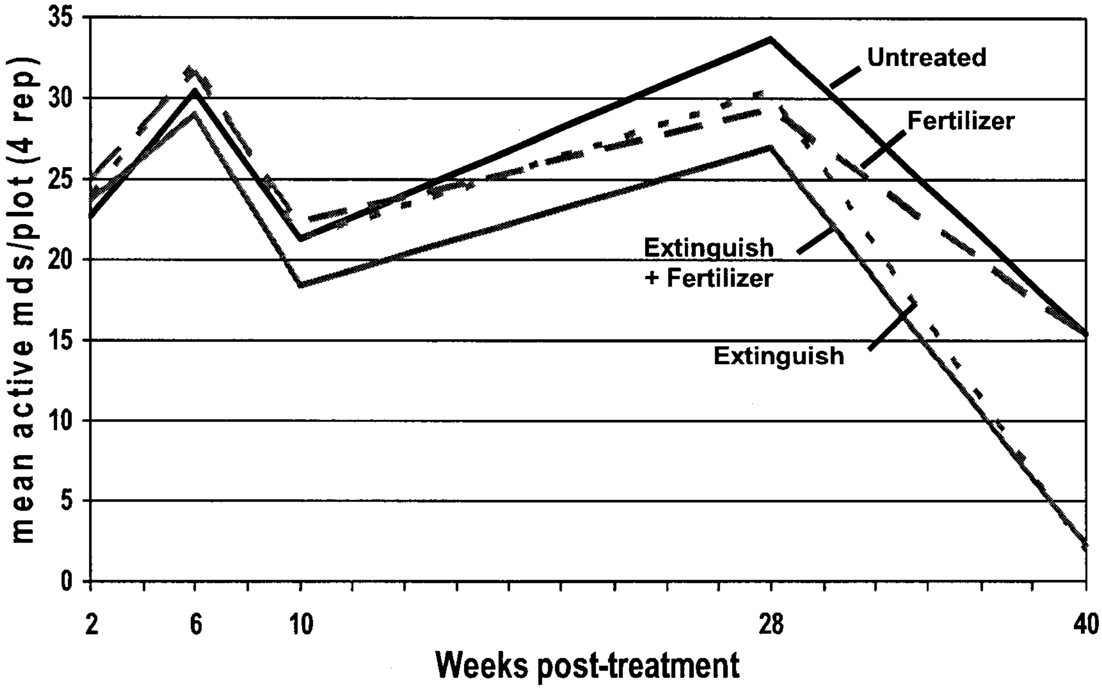


Figure 3. Extinguish + fertilizer mix-and-apply, 2003

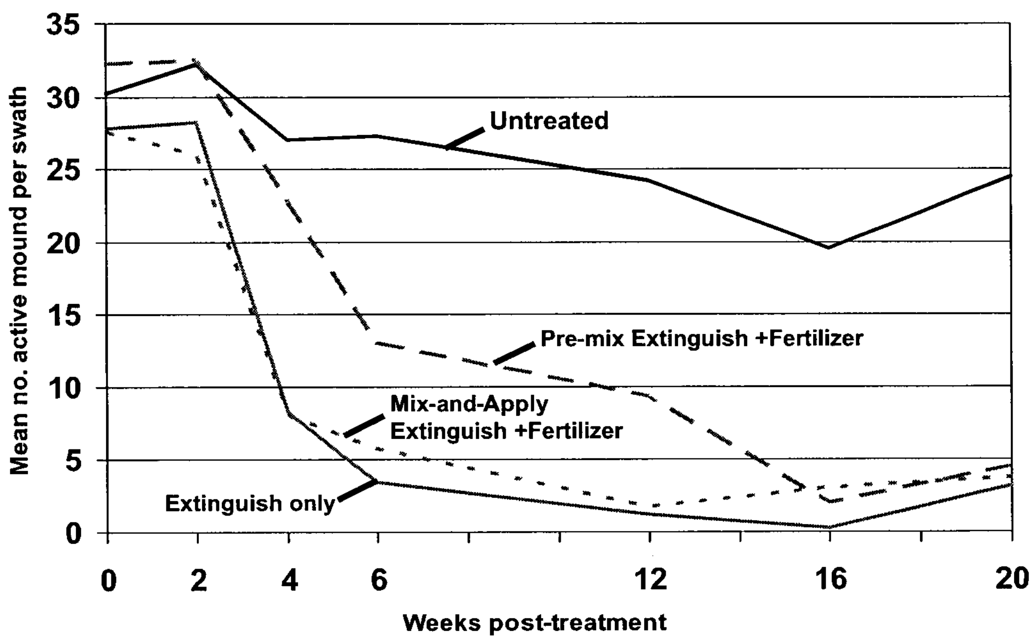


Figure 4. Extinguish + fertilizer mix-and-apply vs pre-mix (2 week), 2004

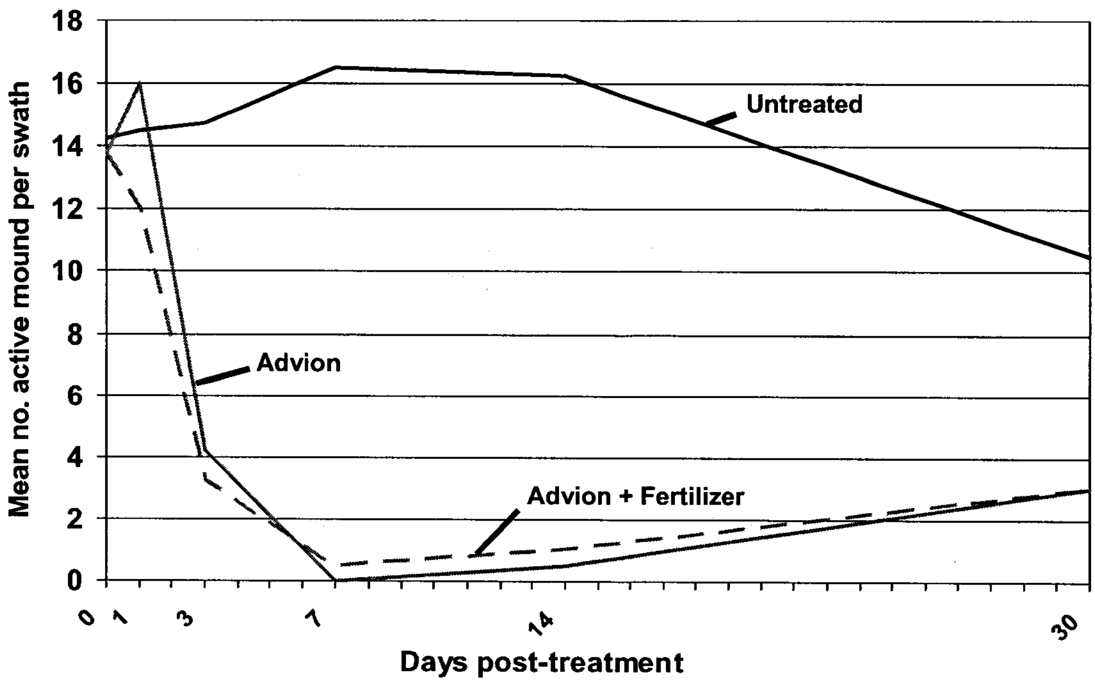


Figure 5. Advion + fertilizer mix-and-apply, 2004

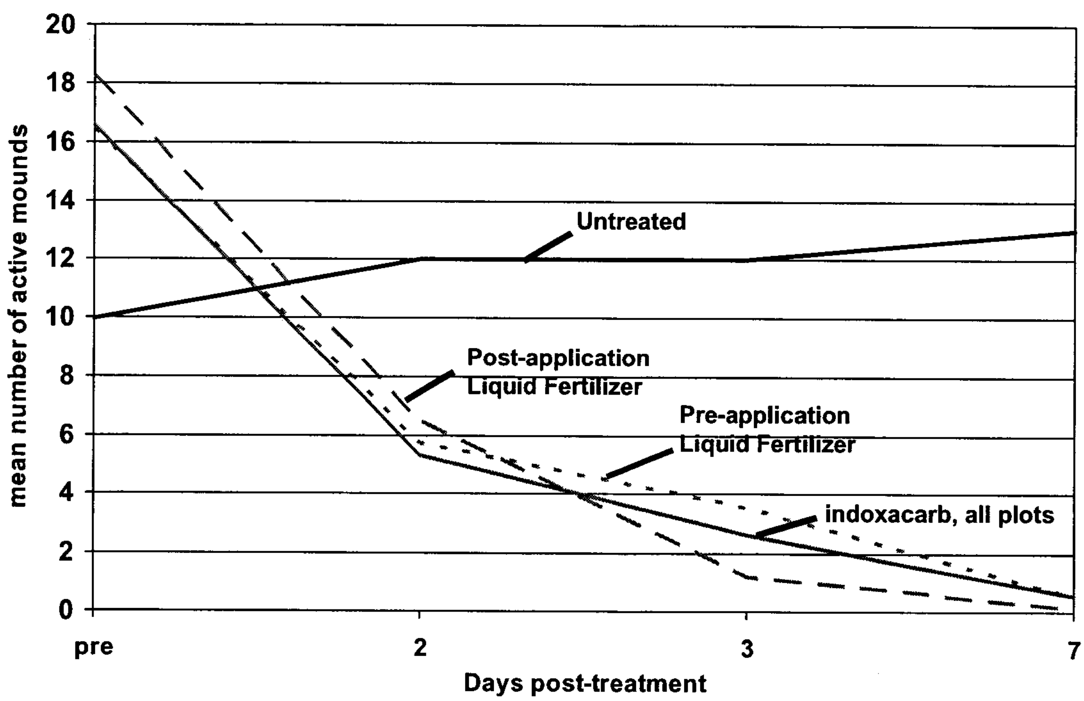


Figure 6. Liquid fertilizer before and after bait, 2004

Report on 2005 ANDE Development Activity: Tast-e-Bait® and Fertibait™ for Use with Imported Fire Ant Control Agents

Timothy D. Birthisel, Technical Development Manager

The Andersons, Inc. Turf and Specialty Group, P.O. 119, Maumee, Ohio 43551

Background about our business in commercial granular pesticide formulations:

The Andersons, Inc. (NASDAQ: ANDE) Turf and Specialty Group has 40-plus years experience as a manufacturer of granular pesticide carriers, with extensive Imported Fire Ant control formulations manufacturing and development experience.

Historically, we are the recognized leaders in corn cob manufacturing technology, having provided corn cob granular carriers used for Mirex Fire ant bait. We were the main provider of the consumer formulations used for broadcast and mound drench treatments, beginning with the Spectracide® Diazinon products first offered by Ciba-Geigy, and continuing to the present day with the leading consumer marketers. Today we employ newer pesticide chemistry and typically use other granular carriers.

The last presentation we did for this conference was to a much smaller audience in Charleston, South Carolina, in 1999, where we introduced our patented DG Lite® dispersible granular carrier. In that presentation we described the superior handling and application properties of that product and its unique one-minute dispersion feature upon contact with water, making it the only disappearing / dispersing non-fertilizer pesticide carrier in the world. We have since learned that this granule is favored due to its property of instantly wetting and sinking when immersed in water, making it especially good for mound drench treatments, where DG Lite stays placed on the surface of the mound while being drenched, and where it quickly releases its toxicant payload directly where it is needed. DG Lite today accounts for tens of thousands of tons of granular carrier use in the here and internationally, much of which is for the Imported Fire Ant control market in the U.S. We thank the Imported Fire Ant Conference organizers for providing a venue to discuss our technology, especially since we are not a significant marketer of end use products in the consumer products market.

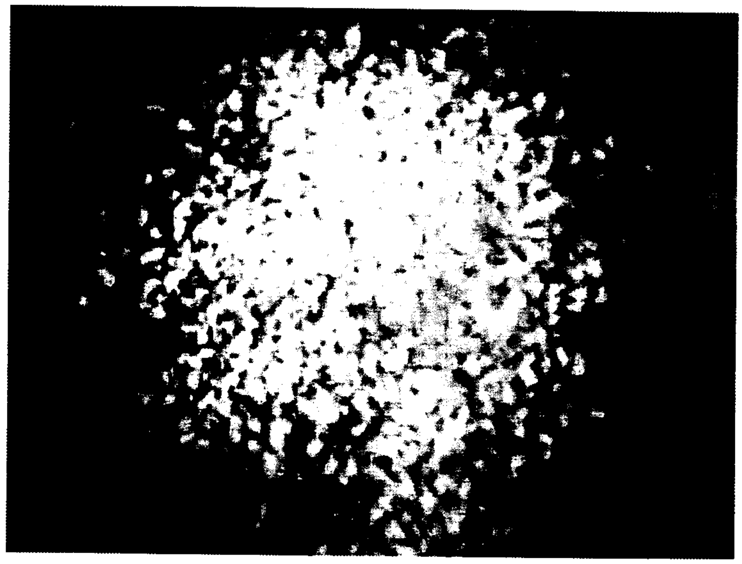
The Andersons Turf and Specialty Group is, however, the recognized leader in granular formulations technology for the U.S. professional turf markets. The leading national branded product lines for the golf and lawn care markets are named Andersons Golf Course, and Andersons Pro Turf. We have decided to leverage our extensive research, formulation, and distribution capabilities into the professional Imported Fire Ant control market under our own brand, and we have filed a patent to cover what we plan to call Fertibait™ technology. This is a unique approach to the broadcast application of insect growth regulators (IGR's) and other pesticides which is intended to provide superior efficacy and convenience to the applicator, allowing the consolidation of two previous operations, spreading fertilizer and insect bait into a single operation. As Charles Barr has presented to this same conference today, this is an attractive and apparently very effective approach to controlling Imported Fire Ants.

However, instead of the user trying to mix his own fertilizer with an existing bait formulation and being concerned with the quality of his mixing operation and the short shelf life of such a mixture (what to do with leftovers?), we want to offer high quality, uniformly pre-blended formulations with storability and reliable performance by design. Our thought is that the typical end user has little interest, and lacks the appropriate equipment required to mix any significant quantities of these granular products together. This is especially true when the expectations of the successful use of both the fertilizer and the insect control bait are at a high level, which is typical among professional applicators.

Tast-e-Bait® and Fertibait™:

The Andersons has been researching the usage of Tast-e-Bait® as an alternative to the typical defatted corn grit bait, which is typically "overloaded" with vegetable oil as an attractant. We have concluded that there are three main attractions to the idea;

1. The logistics are favorable, since the Tast-e-Bait manufacturing site at Okolona, Ohio, is only about a 45 minute drive from our formulating plant in Maumee, Ohio. Besides the obvious freight savings, this proximity allows for just-in-time delivery of the bait to where it is packaged. This avoids needing to stockpile the bait, which can be a problem due its attractiveness to stored grain pests.
2. Tast-e-Bait has the robust characteristics of a granule that can withstand a large scale manufacturing operation, allowing for economical formulation operations.
3. Instead of requiring the heavy loads of vegetable oil that accompany most of the current corn grit bait formulations, Tast-e-Bait appears to have inherent attractiveness and efficacy, presumably due to the significant loading of oils contained internally. The 'greasiness' of the current commercial bait products is illustrated by the photo which was taken of such a formulation which had been manufactured months earlier, allowing the product to "cure," or become apparently drier, following the formulation process. Note the grease stain on the paper towel beneath the small product pile, which formed after only brief contact. This composition moves like a sludge through the formulations equipment, requiring us to remove our product scalping, or cleanup screens, from before the packaging system so that it will flow at all. The flow rate is only half of what we normally see with granules, so we are not mystified that professional users typically must resort to using seed spreaders for applying large quantities, vs. the normal granular application equipment. The exogenous liquid allows roughly 15% of the initial active ingredient to slough off of the granules and onto our manufacturing equipment, which accounts for significant waste generation during manufacturing. We can expect something similar to occur in the packaging materials used to contain the bait, and in the applications equipment used to spread the product. For these reasons, we consider the current typical bait formulation to be undesirable, a veritable "liquid formulation disguised as a granule."



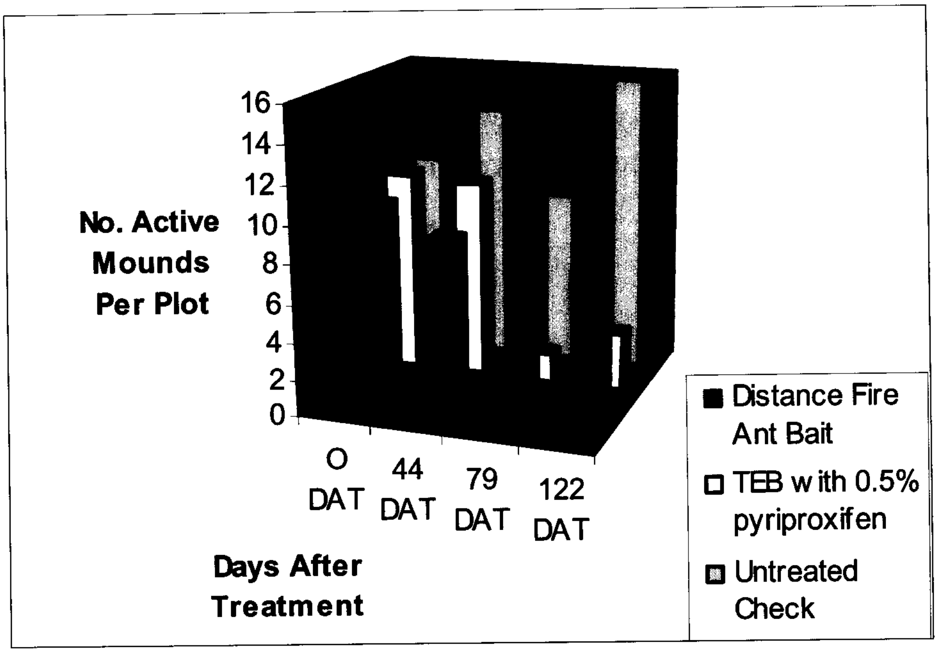
A commercial defatted corn grit formulation allowed to stand on a paper towel
leaves a problematic residue, in which is dissolved a significant part of active ingredient.

Effective Tast-e-Bait formulations don't appear to need such a heavy load of vegetable oil in order to be efficacious, and this lends itself well to the formulation of a stable combination with fertilizer granules. We do not envision that any active ingredient which rubs off of the bait and becomes attached to fertilizer will be attractive to the target insect, so it appears that Tast-e-Bait would be a better fit with the Fertibait concept, since has no excessive oil externally applied. The Andersons expects to obtain a registration for the first Fertibait product we have developed, Fertibait™ with bifenthrin for Mole Crickets, later in 2006.

Our work with Fire Ant Fertibait™ is still underway, and preliminary results are encouraging. In 2005, we contracted with Dr. Rick Brandenburg at NCSU to evaluate both a Tast-e-Bait version of Valent's Distance® Fire Ant Bait, and a couple of Fertibait™ versions, all of which used the same active ingredient and bait application rates per acre (1.5 pounds total bait). The experimental products are all dry, free-flowing formulations.

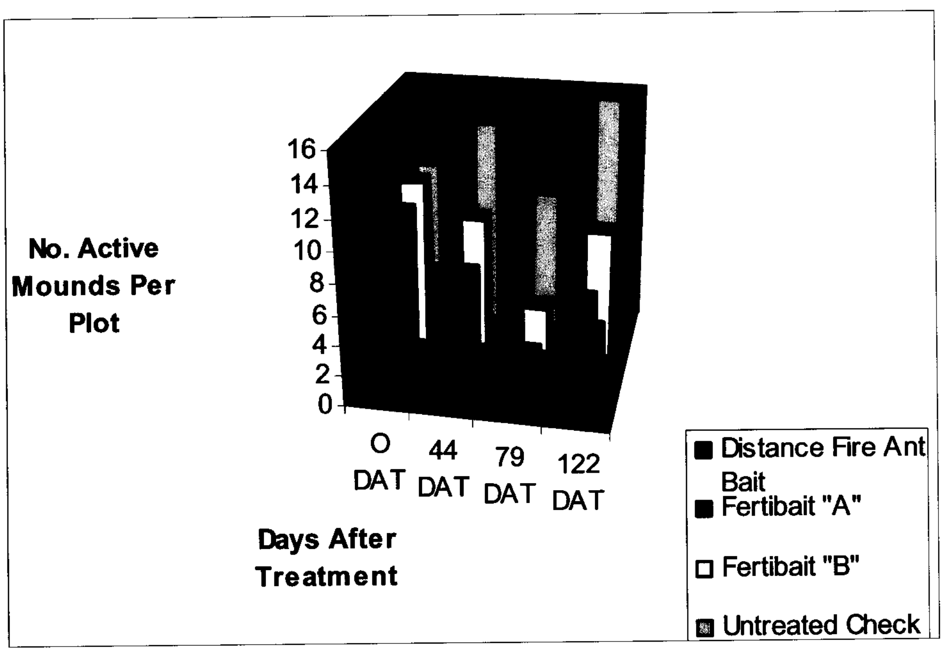
The test protocol was in accordance with the applicable EPA guidelines, and results were encouraging, showing better than or equal efficacy of Distance and the Tast-e-Bait prototype, and between Distance and two experimental Fertibait prototypes. These results appear below.

2005 Small Plot RIFA Bait Trials, Brandenburg NCSU



Applied 6/21 to fence lines and pavement border areas, Fayetteville, NC Office of Public Works.
Remarks: IGR and bait rates / acre were the same. All treatments statistically effective vs. untreated. Small plot sizes likely allowed re-infestation, no mound activity measured. The only statistically significant control differences were at 122 DAT, where Tast-e-Bait performed better.

2005 Small Plot RIFA Fertibait Trials, Brandenburg NCSU



Applied 6/21 to fence lines and pavement border areas, Fayetteville, NC Office of Public Works
Remarks: All IGR and bait rates / acre were the same. All treatments statistically effective vs. untreated. Small plot sizes likely allowed re-infestation, no mound activity measured. The only statistically significant control differences were at 122 DAT, where Fertibait "A" performed better.

Conclusions

Our conclusions from this study are the following:

- pyriproxifen could be good candidate for use with Tast-e-Bait® for Red Imported Fire Ant control
- Preliminary tests support further work with Fertibait™ prototypes for RIFA control.

Development of this promising concept continues in 2006.

Mortality Response of Red, Black and Hybrid Imported Fire Ants to Insecticide-Treated Soil in Laboratory Bioassays

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Abstract: Laboratory bioassays were conducted against Solenopsis invicta, S. richteri and hybrid fire ants to evaluate three insecticides: Talstar, DeltaGard and Dursban. By 0.13 d, a treatment effect was detected. Talstar provided the most rapid mortality: LT50 values were 0.05, 0.13 and 0.21 d for S. invicta, S. richteri and hybrid fire ant, respectively. For all treatments, S. invicta died more rapidly than the other two species. A significant interaction existed between fire ant species and insecticide treatment at 0.13 and 0.21 d. The greater susceptibility of *S.* invicta may be related to its smaller body size.

Introduction

Imported fire ants (IFAs), Solenopsis spp., are pests of agricultural, medical and ecological importance. They were introduced from South America: the black IFA, ***S.*** richteri, in 1918 and the red, S. invicta, around 1930 (Vinson 1997). Biochemical evidence of hybridization between the two species has been reported (Nelson et al. 1980, Vander Meer et al. 1985). Since its introduction, the fire ant has rapidly expanded its range. Fire ants spread to new areas by mating flights or through human activities, such as movement of nursery rootstock, construction equipment, etc., or by unknowingly transporting the mated queens (Vinson 1997). To slow the spread of IFA by artificial means, the USDA enacted a Federal Imported Fire Ant Quarantine in 1958 which regulates the movement of articles, such as soil, plants, grass sod, baled hay and straw, and used soil moving equipment.

Evaluation of chemical insecticides to develop and refine quarantine treatments for IFA control has been an ongoing process (Williams et al. 2001). Laboratory bioassay procedures have been devised to evaluate the effectiveness of insecticides (Callcott et al. 1995). Bioassays are usually performed against S. invicta. Quarantine insecticides may act differently against IFAs other than ***S.*** invicta. This experiment was conducted to compare, in laboratory bioassays, the susceptibility of S. invicta, S. richteri and their hybrid to insecticides available for IFA control.

Materials and Methods

Treatments consisted of DeltaGard® (deltamethrin) at 0.04 lb a.i/100 gal water, Talstar® (bifenthrin) Nursery Flowable at 0.013 lb a.i/100 water, Dursban® (chlorpyrifos) at 0.13 lb a.i/100 gal water and a water-only control. Balled-and-burlapped nursery rootballs were treated by immersing individual rootball in treatment solutions. Soil samples were collected from treated rootballs at 4 weeks post treatment application. Approximately 500 g of soil was collected from each rootball, bagged and stored at 6°C.

S. richteri and the hybrid IFA were collected from Giles and Franklin Counties in south-central Tennessee. S. invicta were collected from South Meridian, Mississippi. An IFA colony

was excavated and transported to the laboratory in 5-gal pails lined with fluon (polytetrafluoroethylene) and covered with lids fitted with metal screen.
Fire ant colonies were maintained at 70±3°F at 14:10 L:D photoperiod and 60% RH in the collection buckets. Each colony was provided with 10% sucrose solution in test tubes plugged with cotton, and frozen field crickets.

The bioassay chambers were constructed of plastic flower pots with labstone bottoms. Each bioassay cup contained 30 g treated soil. Fire ant workers from each colony were sorted in plastic containers lined with fluon. Ten workers were isolated with an electric aspirator in a 5 ml glass vial prior to introduction in each bioassay cup. A square Petri dish half was inverted over each bioassay cup with a 20 g weight to further secure it. Test chambers were placed on moistened cotton towels in wooden trays. Fire ant mortality was recorded at day 0.042, 0.13, 0.21, 0.29 (note: 7 hrs = 0.29 day) and then daily until 100% mortality occurred. Effects of treatment and IFA species were evaluated using ANOVA for CRD (SAS Institute 2002). Probit analysis was used to calculate LT50 values for each insecticide and IFA species.

Results and Discussion

At 0.13 d, the IFA species differed significantly in response to treatments. Talstar provided most rapid mortality for all three IFA species. Talstar provided greater than 80 and 90% mortality of *S. invicta* at 0.13 and 0.21 d post introduction, respectively. *S. richteri* reached 100% mortality at 0.21 d, while hybrid IFA mortality reached 100% at 9 d. Overall treatment effects were significant at 0.042, 0.13 and 0.21 d post treatment. There was a significant interaction between IFA type and insecticide treatment at 0.13 and 0.21 d post treatment. (ANOVA analyses were conducted for data only up to 0.21 d, because by this time 100% mortality had occurred for at least one of the species in one of the treatment groups.)

In the DeltaGard treatment, *S. invicta*, *S. richteri* and the hybrid IFA reached 100% mortality at 10, 14 and 25 d, respectively. Response to Dursban treatment was similar, and significantly slower than the other treatments, for all three IFA species.

LT50 values for *S. invicta*, *S. richteri* and the hybrid IFA were of 0.05, 0.1 and 0.21 d for Talstar; 0.21, 0.82 and 0.51 d for DeltaGard; and 1.45, 1.71 and 1.69 d for Dursban, respectively. *S. invicta*'s greater susceptibility may be related to its smaller body size. Analyses including head capsule width, not completed at the time of the conference, should determine if size had an effect.

Acknowledgements

The authors would like to thank Robert Vander Meer and Michele Hosack for providing assistance in fire ant species identification; Anne-Marie Callcott and Shannon James for thoughtful inputs during this study; Robert Jones for the collection of *S. invicta* colonies, and Sam Ochieng, Nadeer Youssef and Roger Fox for assistance in conducting the study.

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Metaflumizone Trials Against Red Imported Fire Ants in California Using Corn Chips as an Estimate of Ant Abundance

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Metaflumizone, which is in a new class of pesticides from BASF called semicarbazones, was formally introduced in 2005. It is an EPA reduced risk candidate, and impacts the insect's neurological sodium channel to produce "relaxed paralysis" in insects. Due to its rapid effects, we undertook trials of this compound in Calfornia so as to have the product available for fire ant control. We did trials at Lake Elsinore and Coachella, both in Riverside County.

I. Lake Elsinore

There was a RIFA infestation in a new urban development of Lake Elsinore. The ants were living in irrigated turf next to a sidewalk and on a heavily vegetated adjacent slope on the other side of the sidewalk. Although exit holes could be seen in these areas, mounds were not generally visible. We therefore decided to use foraging activity as an estimate of ant abundance. We have used Lays corn chips at other locations as a fire ant bait, and have found it to be a quick and easy way to monitor ant numbers. A corn chip is placed in a 7 dram plastic vial, which is placed horizontally on the ground. After 1.5 hrs the vial is capped and the ants are brought back to the laboratory where they are counted. We set up 14 plots at this location, each 130 x 26 ft. We used 3 formulations of metaflumizone, plus Amdro and 2 control plots. All treatments consisted of defatted corn grit ant baits that were spread at 1.5 lbs/acre. Within each plot, ant numbers were monitored at the centers of the turf area by putting down 6 vials with a corn chip at 10 ft intervals.

Figure 1 and Table 1 show the results of these trials. For this report the metaflumizone trials have been combined. At 7 and 14 days the metaflumizone gave higher reductions in ant numbers than the Amdro. One year post-treatment ant numbers had almost returned to their pretreatment values.

II. City of Coachella

In this city there was a RIFA infestation at a date palm grove. Ants were living near the bases of the trees, but in many areas there was too much debris on the ground to see mounds. There was also a population of ground squirrels living near the trees, and they removed corn chips that we placed on the ground. We therefore devised a bait station consisting of a small plastic cup (3 in diam x 1.5 in h). We cut out the centers of the plastic lids of these cups and attached to the rims ¼ inch mesh metal screening. This screen allowed ants to enter the cup, but prevented animals from removing the chips. We made a small excavation on the ground in which the cup and its wire lid were place horizontally with the opening flush with the ground (Fig. 2). After 1.5 hrs the wire lid was replaced with a plastic lid, and the chip and ants were brought back to the laboratory for counting.

Plots consisted of a 3x3 block of trees. The center tree in each plot was monitored by putting out a cup containing a corn chip at 90 degree intervals 2 m out from the base of the tree. Thus, there were 4 monitors for each plot. In treatment plots the entire 9 tree plot was treated with one of the ant baits. There were 2 control plots that were untreated. Figure 3 shows a plot of the results of these trials. Table 2 shows differences from pre-treatment values for each of the treatments; only the metaflumizone/corn grit bait was still significant after 154 days. Figure 4 shows the grand means for all post-treatment data over the entire experiment. The best result was obtained with the metaflumizone/corn grit, followed by the metaflumizone/Tast-E-Bait and the Amdro. Table 3 shows the pairwise comparisons of these grand means. In conclusion, the metaflumizone compared favorably with the Amdro, acting more quickly and with better results overall.

Days post-treatment	7	14	29	58
Amdro	85	79	93	100
Metaflumizone	95	99	95	88
Control	-60	69	52	94

Tablel. Percent reductions in ant numbers from pre-treatment values at Lake Elsinore. A negative number means that ant numbers increased.

Days post-treatment	7	35	77	112	154
Amdro	**	**	*	ns	ns
Metaflumizone on corn grit	*	*	*	*	*
Metaflumizone on Tast-E-Bait	**	**	**	**	ns
Control	*	*	ns	ns	ns

Table 2. Wilcoxon signed rank test comparison of pre- and post-treatment ant numbers. * = P < 0.05; ** = P < 0.01; ns = not significant.

	Control	Amdro	Tast-E-Bait	Corn grit
Control	--			
Arndro	ns	--		
Tast-E-Bait	*	ns	--	
Corn grit	***	**	*	--

Table 3. Pair-wise comparison of grand means of all post-treatment data (see Fig. 4). * = P < 0.05; ** = P < 0.01; *** = P < 0.001; ns = not significant. "Corn grit" = metaflumizone on corn grit; "Tast-E-Bait" = metaflumizone on Tast-E-Bait.

Figure 1. Average numbers of ants on corn chips at Lake Elsinore trials.

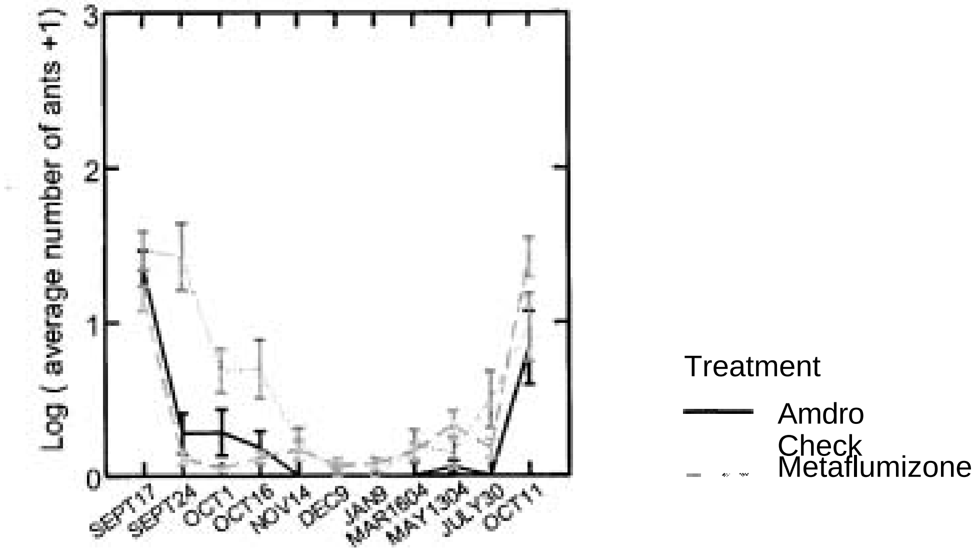


Figure 2. Corn chip inside of plastic cup in small depression on ground.

Figure 3. Average numbers of ants on corn chips in city of Coachella. "Corn grit" = metaflumizone on corn grit; "Tast-E-Bait" = metaflumizone on Tast-E-Bait.

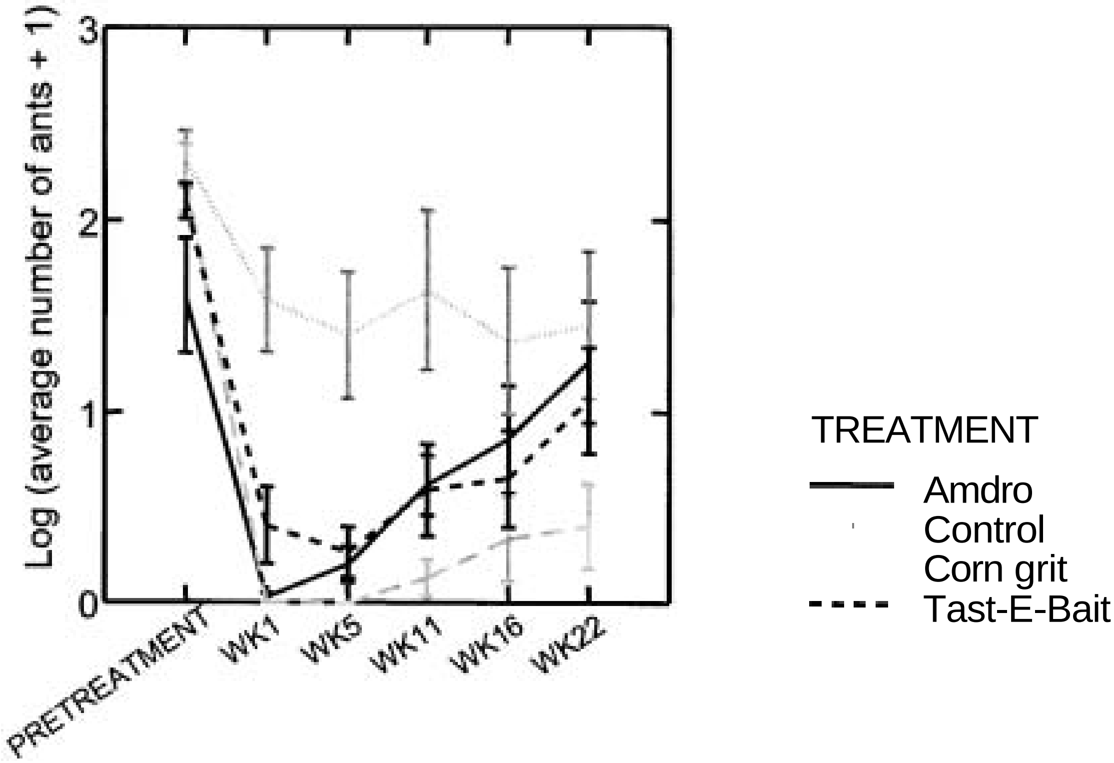
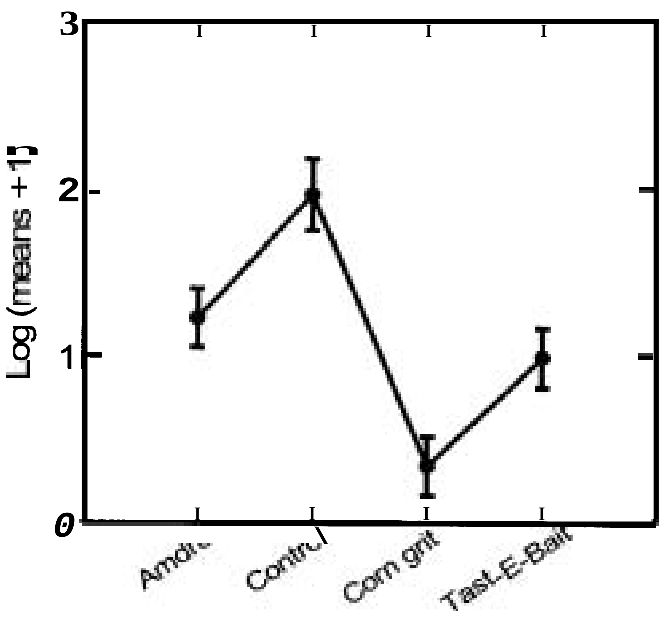


Figure 4. Grand means of all post-treatment data. Means are based on average number of ants on corn chips. Log transformation used to normalize data. "Corn grit" = metaflumizone on corn grit; "Tast-E-Bait" = metaflumizone on Tast-E-Bait.



Fire Ant Management in Poultry Houses

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Abstract:

Fire ants can be a critical factor in poultry management. Foraging in the feed bins, feeding in the houses and stinging the chickens can affect the growth and development of the young chickens through stress. The loss of feed, stinging the workers and the shorting on electrical systems and motors are a burden to the poultry fanner. The implementation of a management scheme for fire ant management on a poultry farm was requested by the poultry farmer, Mr. Billy Gains. Through the implementation of the program the farmer was able to reduce stress on his flock, reduce feed loss, and save on the reduced losses of electrical systems and motors. Other species on ants returned to the environmental system, five species were collected. A two step approach was used to manage the population. Orthene was applied to the mounds against the foundation of the poultry houses and those mounds within 10 feet of the base of the foundation. Extinguish was applied using a Herd spreader to the 17.5 acre farm and dwelling around the poultry houses. Ants were monitored pre application and monthly over a two year period. The program of fire ant management was presented to the poultry farmers at a field day. The city parish government adopted and utilized the program to manage fire ants on the public grounds maintained by the city.

Conclusion:

Fire ants can be effectively managed on a poultry farm through the use of growth regulator baits. The ant population can be reduced so that ants are no longer observed or found trailing into the feed bins or the poultry houses. Fire ants were not getting into electrical equipment and motors reducing the farmers' losses in these areas. The program has shown that fire ants can be kept below threshold throughout the entire farm and residential area by following the program of treating twice a year. Additional cattle and poultry farmers have implemented the program since its completion.

Results **of 2005** Texas Aerial Applicators **Survey**

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There are approximately 90 aerial application businesses in Texas and 42 responded to the Texas Aerial Application Survey focusing on using aerial equipment for the application of fire ant baits. This survey found that 95% of the respondents used fixed wing aircraft and 10% utilized helicopters or both. Also, 95% of the aircraft were equipped with GPS (global positioning system) units, of which 67% were used to locate fields and 97% were used to set swaths. The average maximum number of miles an applicator would travel from the airstrip was 128 miles (range from 0 - 2500 miles with the median and mode = 50 miles). The average charge to apply granular material to an acre of land was $5.61 (range from $2.00 - $12.00 with the median and mode = $5.00). The average minimum area to justify an application was 225 acres (range 25 - 2500 acres, median = 85 acres, mode = 100 acres), with an average minimum cost of $430 (range from $150 - $1000, median = $500, mode = $500). Additional information showed that a majority of the applicators had fire ants in their service area, and were aware of fire ant bait products. Most of them were aware that equipment modifications were required and a large majority wanted additional information on these modifications. Most needed to check on FFA regulations and insurance issues regarding aerial applications to highly populated areas.

Preliminary Observations of Phorid Fly *(Pseudacteon curvatus* Borgmeier) and Black Imported Fire Ant *(Solenopsis richteri* **Forel)** Interactions with High-Speed Videography)

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Abstract

High-speed video recordings of phorid fly and fire ant interactions can easily be made in the field at field mounds. We successfully recorded flying phorid flies and walking fire ants at 10,000 frames per second. This was sufficient to allow later viewing of individual wingbeats of the phorid flies. We could track individual phorid flies for up to 10 seconds at 10,000 frames per second.

Introduction

Due to their potential in controlling imported fire ants in the United States (Graham et al. 2003), phorid flies have been the subject of many intensive studies. Observations of the interactions between fire ants and phorid flies have involved human observers and standard video recordings (Wuellner et al. 2002). Most of this study has been in the laboratory or in artificial habitats. Human visual observations and standard video recordings (around 30 frames/second) are often inadequate for observing the extremely fast flight of phorid flies as they hover around their potential fire ant hosts. In the field with large numbers of phorid flies it is impossible to track individual phorid flies visually or with standard video.

We tested the feasibility of using high-speed video recordings to study interactions between the phorid fly (Pseudacteon *curvatus*), and the black imported fire ant (Solenopsis richteri) in the field in northern Mississippi in Novenmber, 2005. Our goal was to determine if it was possible to capture these interactions in the field with high-speed video.

Materials and Methods

Large black (or hybrid) imported fire ant and phorid populations occur in northern Mississippi not far from the Tennessese line. Our study site was on County Road 202, Lafayette County, Cambridge, Mississippi at the southern boundary of the Holly Springs National Forest and is just north of the Cambridge United Methodist Church. GPS coordinates were: N 34°27.242', W089°22.443'. Field Dates were November 11, 13, 14 and 15. One recording of fire ant/phorid interactions was made per day usually at around 1300 hours.

Our field setup is shown in Figure 1.

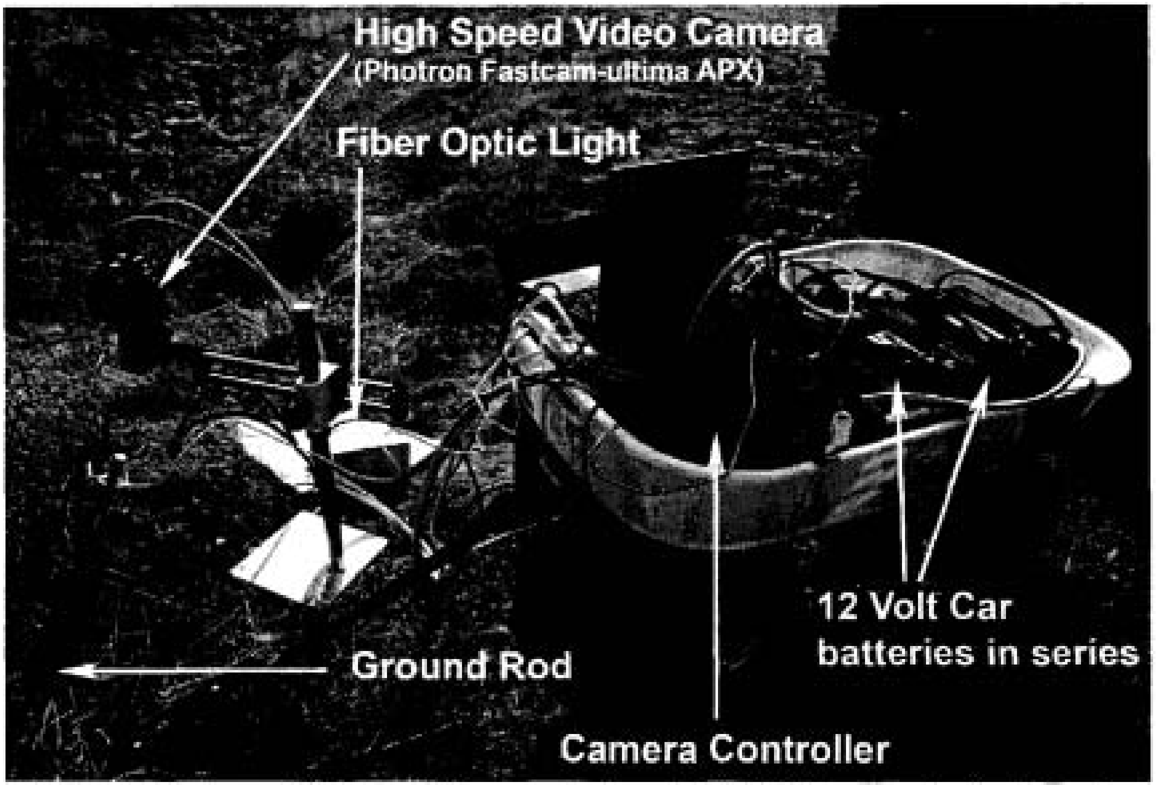

Fig. 1. Field high-speed video set-up (sine-wave inverter not visible). Video camera is situated on a boom stand above a black imported fire ant mound.

The High-speed video camera is the Photron FASTCAM-ultima APX. This camera is capable of 120,000 video frames per second. In this study we captured images at 10,000 frames/second for up to 10 seconds real-time. The camera controller is equipped with 8 Gb of memory in order to store the high speed video. Once capturing was completed in the controller it took several hours to download the controller memory images to a laptop computer through a standard FireWire interface. Power must be supplied with no interruptions during the download or the video will be lost. The camera head was mounted on a boomstand. The lens was a standard Nikon 65 mm Micro-Nikkor macro lens. A cable connected the camera head to the camera controller and a FireWire cable connected the controller to a laptop computer where images could be viewed and captured. Electrical power in the field was supplied by two standard car batteries wired in parallel and attached to a sine-wave inverter (Invertek 1500 Watt Pure Sine Wave Inverter) for clean AC power for the high-speed camera controller and laptop. The entire system was grounded by a four foot long, half inch wide carbon rod inserted into the ground.

Phorid flies were induced to come to fire ant mounds by using a finger or pencil eraser end to poke a small hole in the top of the mound in order to cause fire ants to emerge en masse and thereby attract phorid flies. The camera lens was focused on the emerging ants and video recordings were started once phorid flies were seen around the hole. Recording times varied from less than a second to approximately five to ten seconds. All recordings were at 10,000 frames per second. Recordings were then downloaded to the hard drive of a portable computer hooked up to the sine-wave inverter AC power. This took up to 3-4 hours.

Results and Discussion

At this time of year phorid flies were noticeable but much fewer in number than in the summer. Shade air temperatures during this week were 18.5°C to 29.6°C and phorid flies soon appeared at the disturbed mound (often within a few minutes of mound disturbance). Recordings were made once per day over five successive days. It was difficult to make more than one recording a day because about 10 seconds of video recorded at 10,000 frames per second takes about three to four hours to transfer from the very fast memory of the camera controller to a standard laptop computer through a standard FireWire interface. During this transfer the camera controller must be powered continuously or loss of the image data will result. After this week of warm weather continual cold fronts eliminated all active phorid flights.

Before beginning this study, we were apprehensive about the success rate of actually recording phorid flies interacting with fire ants due to their very fast flight and the low depth of field inherent in any close-up photography. However, high-speed video capture of phorid flies in flight around fire ants was very easy and we were successful at all attempts. Ten thousand frames per second was sufficient to capture individual wingbeats and we could easily track individual phorid flies if they did not fly from the field of view. Luckily, phorid flies often hovered very close to the fire ants, allowing us to follow them for some time, and with both the phorid fly and fire ant in reasonably good focus. Very close-up photography had sufficient resolution to allow one to see details of the attack process. However, pulling the camera back to allow a greater field of view was often desirable in order to track flies and ants for a long time. In one video recording a phorid fly targeted two fire ants and attacked one fire ant twice. At the low population levels of flying phorid fly female adults at this time of year, the fire ants seemed oblivious to their presence despite the fact that the flies were often less than a cm away.

Our goals for 2006 include large numbers of recordings throughout the phorid flight season. We want to record fly and ant interactions at small, medium and large phorid populations. We also want to record during fire ant alate emergence which seems to be the largest attraction of phorid flies to mounds. We will experiment with slower frame rates sufficient to allow following phorid fly activity for longer periods of time.

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Differential Cuticular Chemical Profiles Between Monogynous and Polygynous Red Imported Fire Ant *(Solenopsis invicta)*

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The two social forms, monogyne and polygyne, of Solenopsis invicta, the red imported fire ant, have distinctly different aspects of ecology, physiology, genetics, and some behavioral characteristics such as the aggressive abilities, territorial ranges, and acceptance of winged sexual females. All of these phenomena may be related to the recognition behavior in ant communication which is correlated to different chemicals on cuticle. Cuticular chemicals can be divided into two major groups: hydrocarbons comprised mostly of alkanes, and non-hydrocarbons comprised mostly of alkaloids. In this study, monogyne and polygyne are determined by the number of queens per colony, and also reconfirmed by multiplex PCR with specific primers of ***Gp-9*** alleles. After identifying the social form, cuticular chemicals, both hydrocarbons and non-hydrocarbon, of monogynous and polygynous ants were extracted and analyzed by gas chromatography and gas chromatography-mass spectrometry. Our preliminary result suggested that not only cuticular hydrocarbons but also non-hydrocarbons could be important in ant communication. Using principle component analysis to ordinate those significantly different chemicals didn't show separable clusters. Nevertheless, the PC1 and PC2 axes displayed the monogynous queens were closer to each other than polygynous. For these chemicals have genetic basis, we suggest that lower genetic variation in monogyne was probably by strong aggressiveness to conspecific ants from other colonies. However, the chemicals involved in discrimination behaviors need to be further confirmed in future.

Chemicals Incorporated in Nest Material by Red Imported Fire Ants

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Abstract

Red imported fire ants are believed to incorporate ant-derived chemicals in nesting material. However, only a few chemicals have been identified. One hurdle for such investigation is the interference in chemical analysis from soil-borne chemicals. Ants were found to be able to construct their nests using moistened silica gel as the only building material in the laboratory. This provided an opportunity to identify ant-derived chemicals in nest material without dealing with the interference from soil-borne chemicals. Ants were allowed to build their nest in a device using moistened silica gel as the only nesting material. A similar device with silica gel was used as a control in which no ants were released. Workers built nests by excavating silica gel and making honeycomb-like structures in the device. After building the nests, silica gel samples were collected, extracted with pentane, pyridine or water, and analyzed using gas chromatography – mass spectrometry (GC-MS). Since no food was provided during the experiment, any chemicals which existed in silica gel in the nesting device but not in the control were assumed to be from the ants. Ants seemed to prefer depositing their excreta on the edge of the nesting device, which were mixed with silica gel. Since excreta are believed to contribute to the chemical modification of ant nest soil in the field, excreta samples were also analyzed. All silica gel samples contained cuticular hydrocarbons. Venom alkaloids, phosphoric acid, glycerol, lactic acid, and malonic acid were identified in samples collected inside the nest. Uric acid, 2,6-dihydroxy-9H-purine, urea, glycerol, phosphoric acid, amino acids, and organic acids were found in fire ant excreta.

Introduction

Nest construction by the red imported fire ant, *Solenopsis invicta* Buren alters not only physical properties of the soil, such as infiltration and leaching, but also chemical properties. There is evidence that red imported fire ants incorporated ant-derived organic compounds in the nest soil. Cuticular hydrocarbons were found in the nest soil (Vander Meer, unpublished data, cited in Vander Meer and Lofgren, 1988). It is believed that red imported fire ants can disperse venom alkaloids through the air to the brood surface and mound soil (Obin and Vander Meer, 1985; Storey et al., 1991). Chemicals incorporated in the nest may be critical to social function of an ant colony. Hubbard (1974) found fire ants preferentially dug in nest materials from their own colony. It is likely that chemical cues incorporated into the nest soil by ants themselves affected their digging behavior. A fire ant nest would be an ideal place for the growth of microorganisms considering its regulated moisture and temperature conditions. Ants may constantly face the challenge of pathogenic microorganisms in their environment. A mechanism to suppress the growth of detrimental microorganisms inside the nest may contribute to colony fitness. Antimicrobial agents incorporated into nesting material may serve this purpose. Indeed, venom alkaloids of fire ants are antimicrobial agents (Blum, 1988).

Chemical analysis of organic compounds in soil is problematic, because soil is a complex biomaterial. In a preliminary study, I found that red imported fire ants can construct nests using moistened silica gel as the only building material under laboratory conditions. This provided an opportunity to examine the ant-derived chemicals in the nest material without interference from any soil-borne chemicals. The objective of this study was to establish a more detailed profile of ant-derived chemicals in the nesting material by analyzing nests formed with silica gel.

Materials and Methods

Two nest building scenarios, queen-less subset colonies and queen-right intact colonies, were set up in the laboratory using silica gel (35-60 mesh) as the only nest building substrate. The nesting devices for both nesting scenarios are illustrated in Fig. 1. The device for queen-right colony consisted of one plastic tray (20 cm × 6 cm) and a 500-ml square bottle right underneath it. There were five 3-mm access holes between the tray and the bottle. Inside wall of the tray was coated with Fluon. The bottle was filled with moistened silica gel (50% water). The nesting device for queen-less scenario was similar except smaller in size (Fig. 1). A petri dish and a capped Wheaton liquid scintillation vial were used with only one 3-mm access hole between the Petri dish and the vial. The inside wall of the Petri dish was coated with Fluon.

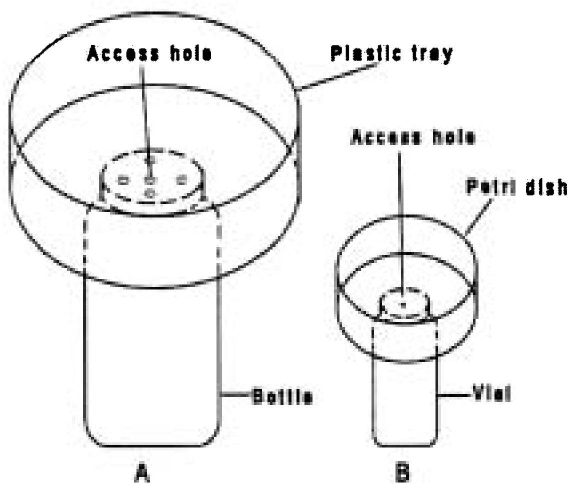

Figure 1. Nesting devices for queen-right colonies (A) and queen-less subset colonies (B).

Matured colonies were used, evidenced by the presence of brood and alates. Four colonies were provided for the queen-right colony scenario and seven colonies were used for queen-less scenario. The whole colony was used for queen-right scenario (21 to 45g); whereas in the queen-less scenario, 1.25 to 1.50g ants [12.21 to 18.23% brood and 8.20 to 10.02% alates (male + female)] was used for each nesting device. Ants were released in the plastic tray or the Petri dish. All experiments were conducted at 21-27 °C and relative humidity between 70 and 85%. Controls were established in the same way as described above except no ants were released in the devices.

After two weeks, the silica gel samples from nesting devices were transferred to separate aluminum pans. A Petri dish with a 0.5 cm entrance hole on the side was placed upside down in the pan. After workers collected brood and transferred them into the Petri dish, silica gel in the pan was then collected. Samples were stored at -15 °C in a glass beaker which was sealed with

Teflon tape. Only fresh samples stored in refrigerator for less than two days were used for chemical analysis.

Some silica gel particles in the plastic tray and Petri dish turned brown, most likely due to ant excretion. The colored silica gel samples (each consisted of 10-15 silica gel particles) were directly derivertized with 50μl N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) in 150μl pyridine at 60 °C for 6 h without any pre-extraction. The uncolored silica gel in plastic tray / Petri dish and all samples in bottle/vial (each sample consisted of 2.5 to 3.0 g silica gel) were subjected to three different solvent extractions: pentane, pyridine, and water (6 × 3 ml). Pentane and pyridine extractions were concentrated to 200μl and no derivertization was conducted. The water extraction was dried in a freeze drier and then derivertized using 50μl BSTFA in 150μl pyridine.

The presence of cuticular hydrocarbons and venom alkaloids in nesting material was confirmed by comparing chromatograms and mass spectra of silica gel samples to those of worker pentane extractions. Cuticular hydrocarbons and venom alkaloids in worker pentane extraction were separated using a silica gel column, The column consisted of a 14.6 × 0.6 cm disposable pasteur pipette filled with 5 cm 35-60 mesh silica gel and the tip blocked with a piece of glass wool. Cuticular hydrocarbons were eluted with the first 8 ml hexane and venom alkaloids were then eluted with the 4 ml pyridine. Identification of all other chemicals was achieved by comparing the GC retention time and mass spectrum of the targeted compound to those of a chemical standard. If the chemical standard was not available, the identification was achieved through mass spectra library search and marked as tentative identification. A Varian GC-MS system was used for this study. It consisted of a CP-3800 gas chromatograph with a DB-1 capillary column (30 m × 0.25 mm i.d., 0.25 μm film thickness) and a Saturn 2000 mass selective detector. The GC temperature program was as follows: initial temperature was 50 ˚C, held for 1 min., increased to 240 ˚C at a rate of 20 ˚C /min, and held for 29.5 min. The split ratio was 1:10, injection temperature was 250 ˚C, and transfer line temperature was 270 ˚C. The mass spectrometer was operated at 70 eV in the electron impact mode.

Results and Discussion

Cuticular hydrocarbons were found in all samples and the retention times and mass spectra of cuticular hydrocarbons exactly matched to those of worker body extractions (Fig. 2). Samples collected inside the bottle and vials contained lactic, malonic, and phosphoric acids as well as glycerol and venom alkaloids (Fig. 3 and 4). Uric acid, 2,6-dihydroxy-9H-purine, urea, glycerol, phosphoric acid, amino acids, and organic acids were found in fire ant excreta (Fig. 5).

In agreement with the literature (Vander Meer, unpublished date, cited in Vander Meer and Lofgren, 1988; Obin and Vander Meer, 1985; Storey et al., 1991), fire ants incorporated cuticular hydrocarbons and venom alkaloids in the nest materials. Uric acid and urea were found in all brown silica gel particles. Uric acid and urea are most likely excretory products of fire ants; however, urea has never been reported as a nitrogenous excretory product of red imported fire ants. This raises very interesting questions about the nitrogenous excretory mechanism of red imported fire ants: How is the concomitant excretion of uric acid and urea related to the success of fire ants and whether amino acids and nitrogenous acids, such as 2-quinolinecarboxylic acid, are also excretory products? Apparently the accumulation of uric acid and urea may contribute to the vigorous vegetation commonly found around fire ant mounds.

Glycerol was found in the nesting material and excreta. Glycerol is a humectant which promotes retention of moisture. Incorporation of glycerol in nest material would definitely affect the moisture balance inside the nest. Glycerol may contribute to the micro-climate regulation by

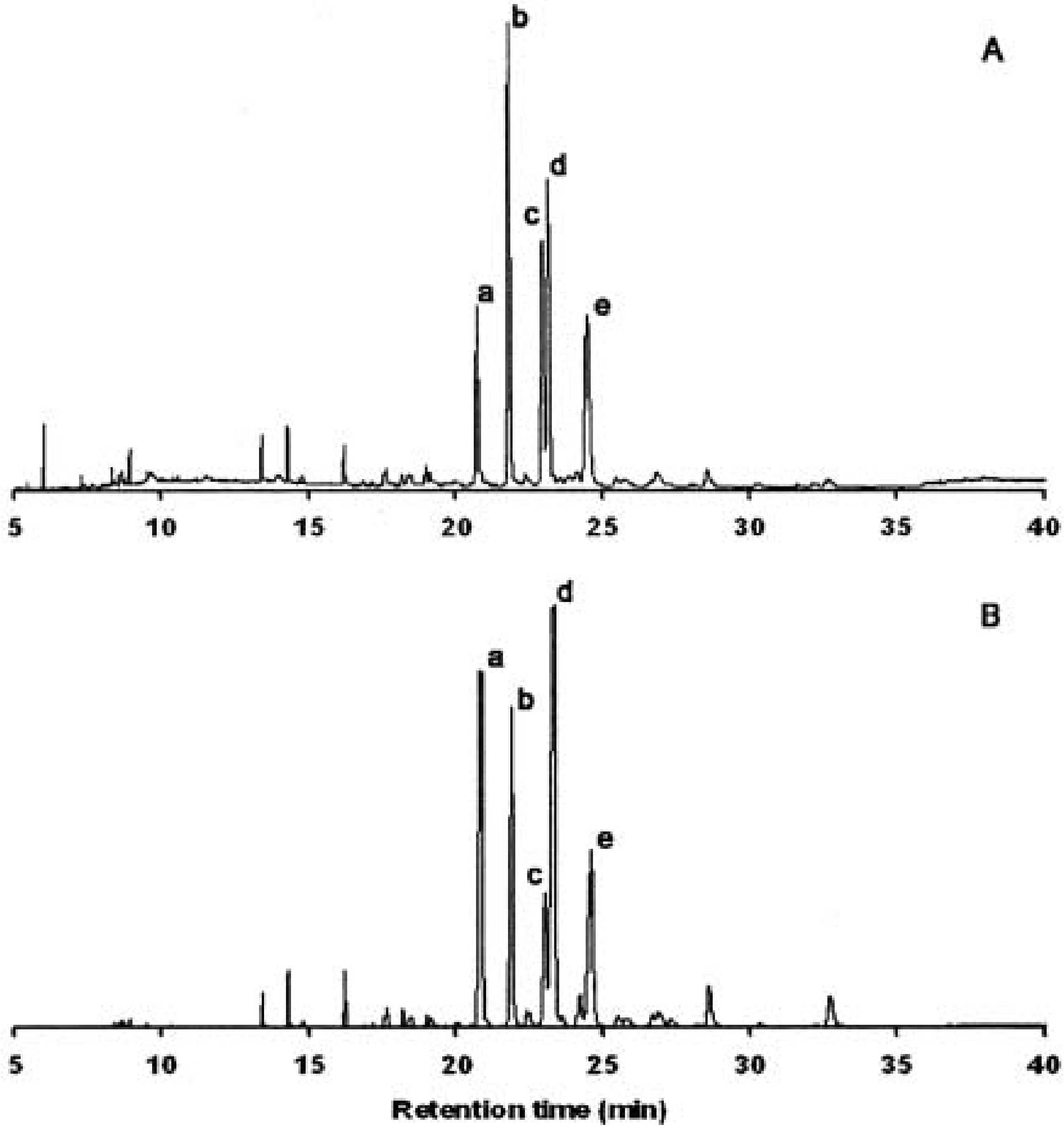

Figure 2. Typical GC-MS total ion chromatograms of pentane extractions of silica gel samples (A) and cuticular hydrocarbons extracted from workers (B). Cuticular hydrocarbons were detected in all silica gel samples. Peak assignments: a: n-heptacosane; b: 13-methylheptacosane; c: 13, 15-dimethylheptacosane; d: 2-methylheptacosane; e: 3,9-dimethylheptacosane (Nelson et al., 1980; Obin, 1986).

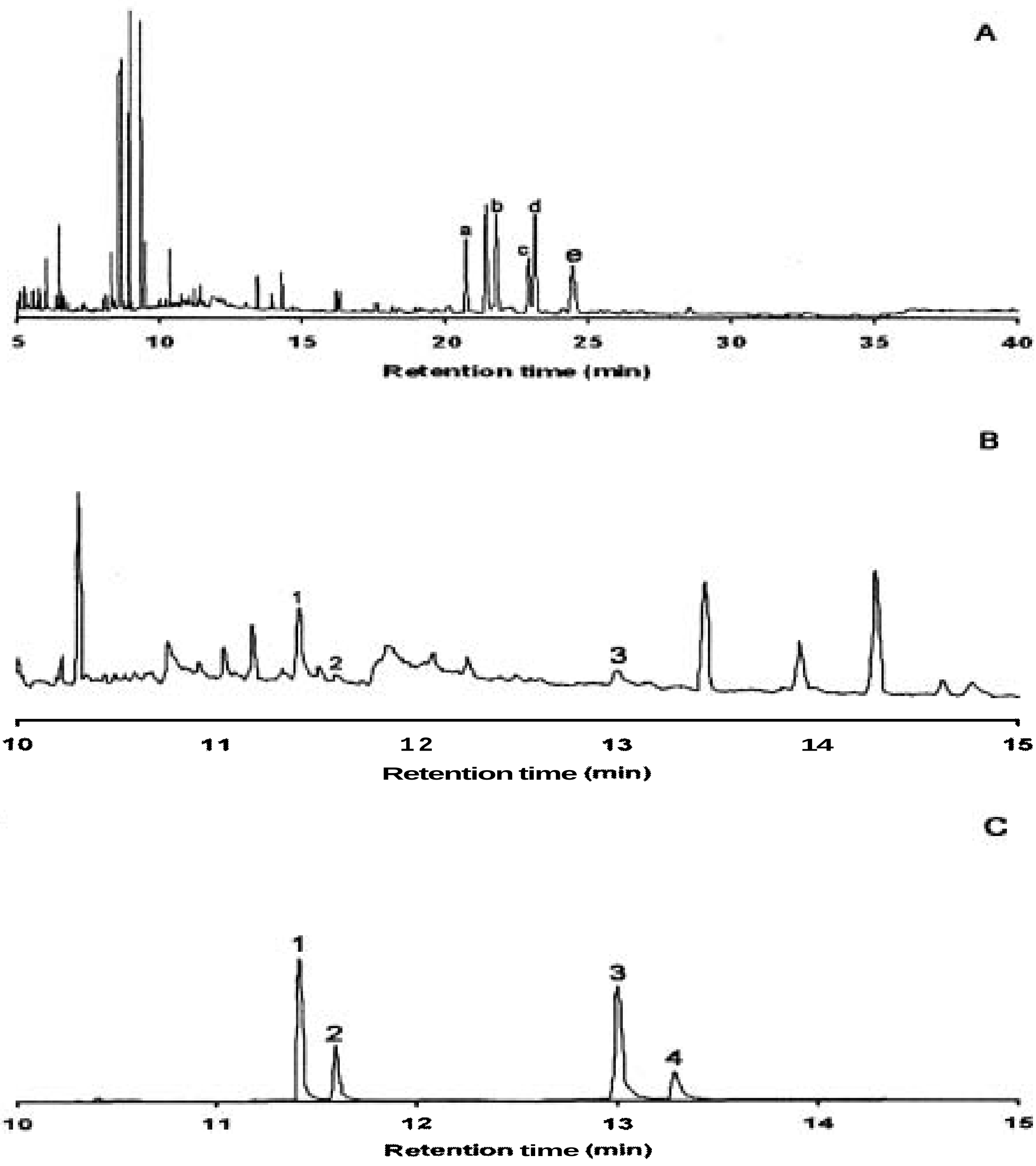

Figure 3. Typical GC-MS total ion chromatograms of pyridine extractions of silica gel samples collected inside the nest (A) and venom alkaloids of the worker extraction (C). Chromatogram in (A) for the region 10 – 15 min was expanded to show the peaks of venom alkaloids. Peak assignment: a: n-heptacosane; b: 13-methylheptacosane; c: 13, 15-dimethylheptacosane; d: 2-methylheptacosane; e: 3,9-dimethylheptacosane (Obin, 1986); 1: trans-2-methyl-6-(cis-4-trideceny1)-piperidine; 2: trans-2-methyl-6-n-tridecylpiperidine; 3: trans-2-methyl-6-(cis-6-pentadecenyl)-piperidine; 4: trans-2-methyl-6-n-pentadecylpiperidine (Ross et al., 1987).

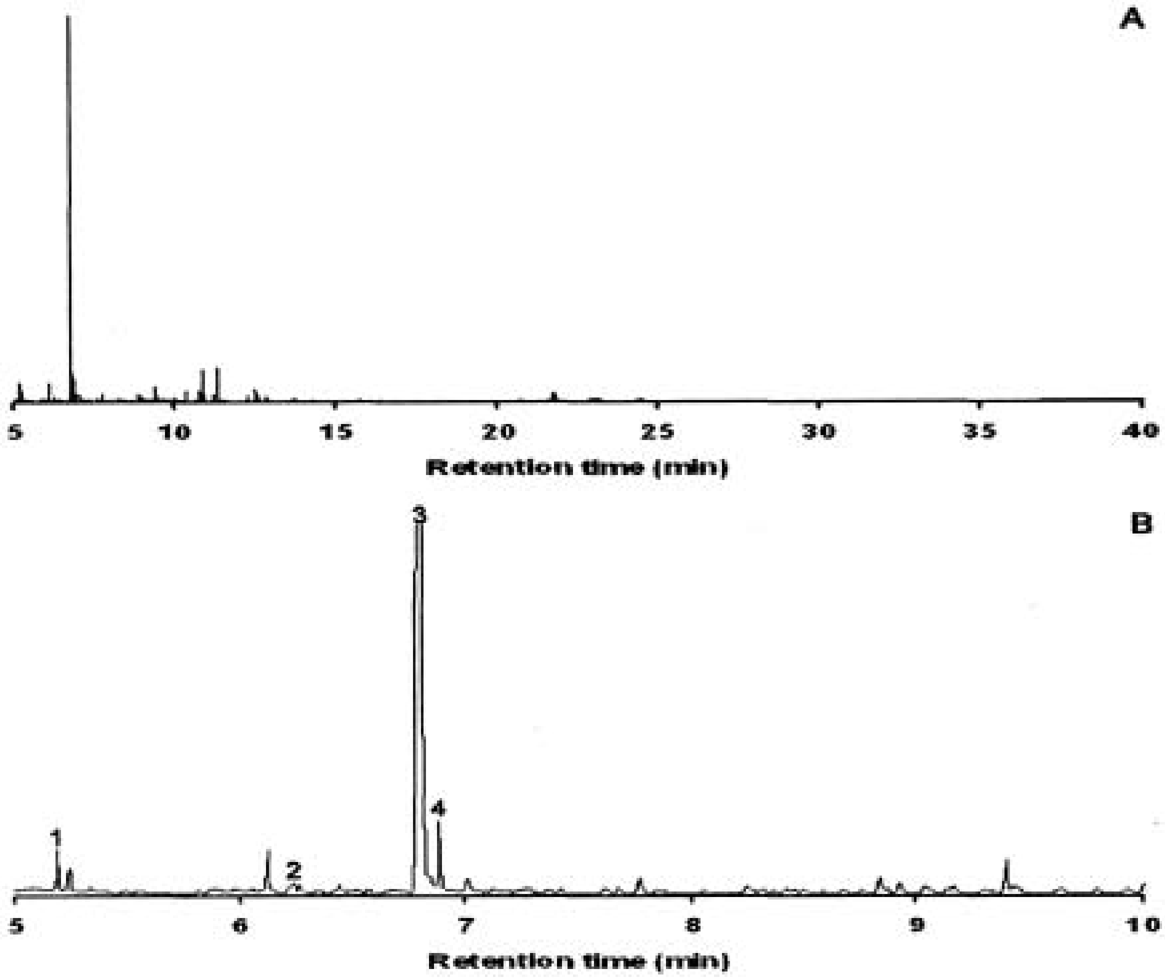

Figure 4. Typical GC-MS total ion chromatogram of water extractions of silica gel samples collected inside the nest **(A).** The chromatogram in (A) for region 5 – 10 min was expanded to show the identified peaks. Peak assignment: 1: lactic acid-tms; **2:** malonic acid- **tms**; **3:** phosphoric acid- tms; and 4: glycerol- tms.

retaining moisture in the nest substrate. This becomes more important when the environmental humidity is low, such as during the dry season. However, more research is needed to test this speculation.

Since excreta was believed to be a source of element enrichment in mound soil of red imported fire ants (Herzog et al., 1976; Green et al., 1998), any organic compounds in the excreta are also likely to be accumulated in the mound. A series of organic acids existed in fire ant excreta and lactic acid and malonic acid were detectable in water extractions of silica gel inside bottles and vials. Organic acids are well-known antimicrobial agents (Cherrington et al., 1991). They can affect the microorganism communities inside the nests, which in return may impact the health of fire ant colonies.

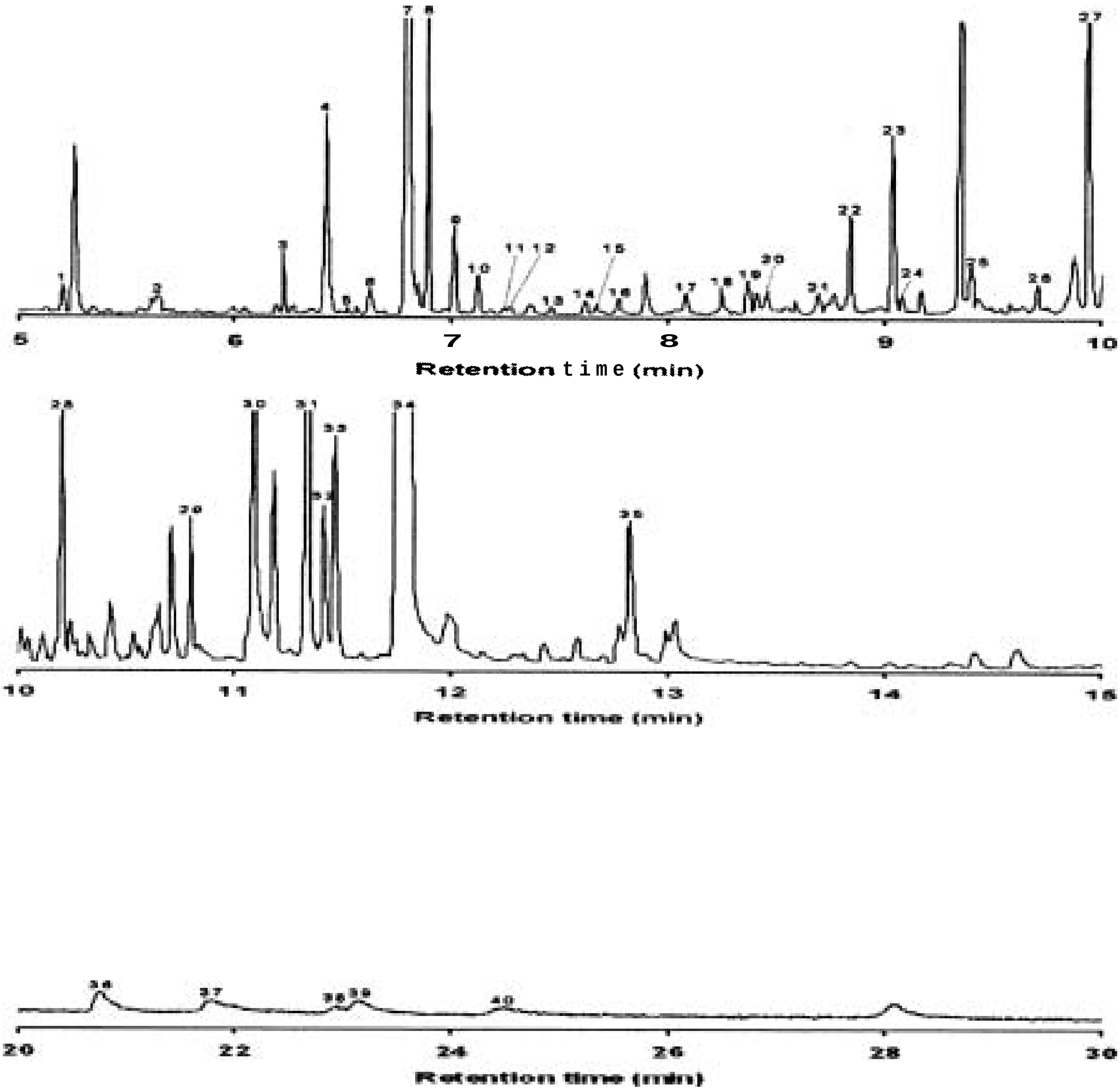

Figure 5. Typical GC-MS total ion chromatograms of colored silica gel particles (excreta). Chromatograms for region between 15 to 20 min and 30 – 40 min were not shown because no peak was identified. Peaks assignment: 1: lactic acid-tms; 2: glycine-tms; 3: malonic acid-tms; 4: urea-tms; 5: benzoic acid-tms; 6: N-acetylglycine-tms; 7: phosphoric acid-tms; 8: glycerol-tms; 9: succinic acid-tms; 10: 2-ethylmalonic acid-tms *; 11: glyceric acid-tms *; 12: fumaric acid-tms *; 13 serine-tms; 14: methylmaleic acid-tms; 15: L-threonine-tms; 16: 3-methylglutaric acid-tms; 17: parabanic acid-tms *; 18: malic acid-tms: 19: 5-oxo-proline-tms *; 20: aspartic acid-tms; 21: 2,3,4-trihydroxybutyric acid-tms *; 22: 2-ethyl-3-ketohexanoic acid-tms *; 23: glutamine-tms; 24: phenylalanine-tms; 25: suberic acid-tms; 26: aconitic acid-tms *; 27: azelaic acid-tms; 28: propanetricarboxylic acid-tms*; 29: tyrosine-tms; 30: 2,6-dihydroxy-9H-purine-tms *; 31: hexadecanoic acid-tms; 32: 2-quinolinecarboxylic acid-tms; 33: gluconic acid-tms; 34: uric acid-tms; 35: stearic acid-tms; 36: n-heptacosane; 37: 13-methylheptacosane; 38: 13, 15-dimethylheptacosane; 39: 2-methylheptacosane; 40: 3,9-dimethylheptacosane.
*: tentative identification

Knowledge on nest chemistry is important in understanding the behavior and chemical ecology of a nest-building ant species. The technique used here may be useful in establishing profiles of ant-derived chemicals in the nests for other mound-building ant species.

Acknowledgments

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Fire Ant Repellents from Plant Extracts

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Introduction:

Many plants have obtained compounds with anti-insect properties during the course of ecological interactions with insects. These natural products can function like other synthetic pesticides, but have low mammalian toxicity and can rapidly degrade in the environment, and thus have broad applied prospects.

So far more than 6,000 plant species have been tested, about 2,500 of which exhibit pest control activity. Compounds extracted from plants have been discovered with activity against cockroach, leaf worm, hopper, beetles, aphids, borers, mosquitoes, ticks, caterpillars, termites, flies, and locusts. Few plant extracts have been tested on fire ants that are major pest insects in many countries including the United States.

The goal of this research was to look for plants containing fire ant repellent compounds and to lay a foundation for further developing some plants into commercial biopesticides.

Materials and Methods:

Selection of Plants: It is estimated that at least 300,000 different kinds of plants occur in the world. To narrow the scope, we focused on plants that would emit a strong odor when shredded, or exhibited repellent effects to other insects (e.g., mosquitoes), or have been known to contain feeding deterrent compounds because fire ants are omnivorous, consuming diversified kinds of plant resources like plants leaves, bark, nectar, sap, fruit, roots, and stems. Based on this idea, we selected 34 plant species belonging to the families Amaryllidaceae, Apiaceae, Asteraceae, Cupressaceae, Geraniaceae, Lamiaceae, and Poaceae.

Source of Plants: Horticultural products were provided by Burgess Falls Nursery (Tennessee), Mary's Greenhouse (Tennessee), Mountain Valley Growers (California) and Supermarkets. Plants were grown in a greenhouse (Fig. 1) at the Tennessee State University, Otis L. Floyd Nursery Research Center (NRC), with being watered regularly and fertilized at times. Short residual insecticides were applied, when needed, to control greenhouse pests. Plants were not used for two weeks after an insecticide treatment to allow time for the chemical to degrade.

Figure 1. Plants for repellent tests in a green house

Preparation of Plant Extracts: The seeds, fruits, leaves, needles, twigs, roots, flowers, bark, and stems of the plants or whole plants were chopped, shredded or grinded using a powerful machine such as a food processor. Between preparations of different plant extracts, the food processor was washed thoroughly with soap and water and then rinsed in ethanol. The ethanol was allowed to evaporate before the next preparation.

Test of Plant Extracts: The following steps were used to test plant extracts: (1) Four hotdog pieces were placed on the center of a rounded wooden board with a diameter of about 7 cm, and then plant extracts were applied evenly to the outer edge of the upper surface on the board; hotdog pieces were selected as the most attractive baits based on preliminary testing among pretzel, potato chips, premium chunk light tuna, and cooked pork meat. The hot dog brand used was Franks and Dinner Delights made with chicken, pork, and beef. (2) Four replicates were prepared for each treatment. The control treatment consisted of a board with only hotdog pieces and no plant extracts. (3) The treatments were placed near fire ant mounds in the field. (4) The test site was in the open area on the north-bound side of the Highway 111 in Hamilton County, Tennessee. Trials were conducted on both cloudy and sunny days, but an umbrella was used to shade test zones on sunny days. (5) The numbers of hybrid imported fire ant workers (Solenopsis richteri x invicta) that came to hotdog pieces were recorded at intervals of 10, 30, 60, 120, and 180 minutes, only workers that gathered on the hotdogs were counted. The number of ants on the boards or on the plant extracts was not included.

Determination of Repellence: The degree of repellence of plant extracts was determined by comparing the ratio of ant response to the treated-versus non-treated board using the formula (Metzger 1930): Repellence value (M) = [(Bait + Repellent)/Bait] x 100. Plant extracts are arbitrarily considered repellents when $M < 25$; attractants when $M > 100$; and neither repellents nor attractants when M is between 25 and 100 (Metzger 1930). The obtained M-values need to be compared using paired t-test analysis ($a = 0.05$).

Results and Discussion

Among the tested 34 plant species tested, five (#10, #11, #12, #23, and #26) exhibited distinct repellency (see Table 1), and two (#21 and #28) need to be tested again to determine if these plants demonstrate repellent properties or not.

The plant species 1, 10, 11, 12, 15, 17, 18, 19, 20, 21, 22, 24, 25 and 29 have a strong odor to the investigator. Among these 'strong-odored' plants, three (#10, #11, and #12) exhibited repellent activity to fire ants, and eleven did not (#1, #15, #17, #18, #19, #20, #21, #22, #24, #25, and #29). The perceived 'strength' of an odor to the investigator is not a valid determinant of whether specific odors may play a role in repelling fire ants.

Plant species 3 has been tested twice, on 8 June 2005 and 8 September 2005. The results of the two tests are contradictory, with 8 June demonstrating no repellency and the 8 September demonstrating repellency. This may result from differences in weather conditions, concentration of active chemical constituents in plants at different times, in the sources of the plant material, or other unknown reasons. Further studies are needed.

Pant species 31 did not exhibit any repellent activities although volatiles from the preparation of plant extracts were irritating to the investigator's eyes. Plant species 18, a member of the genus Tagetes, also did not exhibit repellent properties to fire ants, although there are reports that Terthienyl (Fig. 2) and 5-(3-buten-1-ynyl)-2,2'-bithienyl (Fig. 3) isolated from the Tagetes are toxic to some insects.

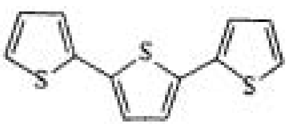

Figure 2. Terthienyl

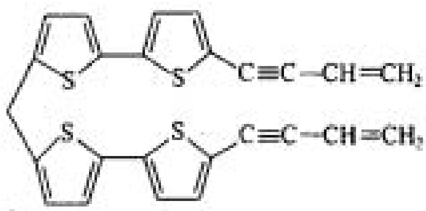

Figure 3.5-(3-buten-1-ynyl)-2,2'-bithienyl

Finally, the efficacy of the plant extracts tested and the foraging activity of fire ants were often influenced by temperature, sunlight, rain, and wind conditions. Therefore, it is necessary to repeat the repellency tests with promising plant extracts to definitively establish that a plant does or does not have fire ant repellent activity. Ultimately, the actual compounds with repellent activity must be isolated and identified. The actual names of plants identified as repellents in this study will be released in the future after we have successfully isolated the active compounds.

Table 1. Results of the tests of the plant extracts against hybrid imported fire ants

Plant	Range of the number of fire ants									
Spp	10 minutes		30 minutes		1 hour		2 hours		3 hours	
	Treat/Cont		Treat/Cont		Treat/Cont		Treat/Cont		Treat/Cont	
*10	0--0	1--5	1--2	2--6	1--2	11--19	2--3	12--26	1--2	10--23
*11	0--0	2--5	0--0	2--8	0--0	8--24	0--0	10--20	0--0	37--65
*12	0--0	4--14	0--0	12--26	0--0	10--20	0--0	13--27	0--0	20--38
*23	0--0	2--4	2--5	13--36	2--5	17--33	5--6	20--32		
*26	0--0	14--29	1--2	53--74	0--2	64--87	0--2	52--123		

Notes: The symbol "*" indicates plant extracts which exhibit distinct repellency.

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The Vegetarian Side of Parasitic Phorid Flies: Use of Non-Host Food by *Pseudacteon tricuspis*

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Abstract

In addition to host feeding, adults of many parasitoid species also use non-host (plant-derived) foods (e.g., nectar and honeydew) as energy sources. This vegetarian side of the diet of parasitoids could have direct and indirect effects on their fitness. The phorid fly, *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) has been released in many parts of southern United States for biological control of red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). However, little is known about the nutritional ecology and foraging behavior and of phorid flies and the impact of sugar feeding on their lifespan. We investigated the effect of sugar feeding on the survival of P. *tricuspis* along with temporal patterns of nutrient accumulation and mobilization in flies provided different diet regimes. Flies fed 25% sucrose continuously throughout their lifespan have greater longevity (mean ± SE longevity: female = 7.9 ± 0.8 days, male = 8.9 ± 0.9 days) than completely starved (provided no water and no sugar solution) flies, sugar starved (provided water only) flies, or flies fed sugar solution only on their first day of adult life. Completely starved flies rarely lived beyond one day. Provision of water increases longevity by 2 days, and one full day of sugar meal further increases longevity by an additional 1-2 days. There was no significant effect of sex on longevity. Adult *P. tricuspis* emerge with no gut sugars, and only minimal amounts of body sugars and glycogen. While the levels of body sugars and glycogen decline gradually in sugar-starved flies, a single day of sugar feeding resulted in the accumulation of maximum amounts of gut sugars, body sugars, and glycogen. High levels of these nutrients are maintained in female and male phorid flies feeding sucrose continuously over the observation period, whereas nutrient levels decline in flies fed only on the first day of life, beginning 1 day postfeeding. Female and male P. *tricuspis* emerge with an estimated 12.3 ± 2.3 μg and 7.2 ± 1 μg lipid reserves per fly, respectively. These teneral amounts represent the highest lipid levels detected in adult flies, irrespective of their diet and are maintained over the life times of sucrose-fed female and male flies, but declined steadily in sugar -starved females. These data suggest that adult P. *tricuspis* are capable of converting dietary sucrose to body sugars and glycogen, but not lipids. We also examined the effects of cotton aphid honeydew and buckwheat floral nectar on the lifespan of P. *tricuspis.* Adult *P. tricuspis* are able to utilize floral nectar from buckwheat and honeydew produced by cotton aphids with increase in longevity and body nutrients, suggesting that availability of supplemental sugar sources near its release sites may enhance the efficacy of P. *tricuspis* as a biological control agent of imported fire ant.

Host Specificity and Transovarial Transmission of *Vairimorpha invictae*

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Summary

Vairimorpha invictae is a microsporidian pathogen of red imported fire ants, Solenopsis invicta. Interest in this pathogen as a potential biological control agent of fire ants stems from shorter survivorship of starved infected adult workers (Briano and Williams 2002) and colony reductions from natural infections in field sites in Argentina (Briano 2005). In addition there are anecdotal reports of faster colony decline with simultaneous infections of *V.* invictae and Thelohania solenopsae, another microsporidian fire ant pathogen. Approval to release *V.* invictae as a biological agent in the U.S. will require, in part, a determination of its host specificity. Examinations of field colonies or ants foraging on food lures in Argentina and Brazil resulted in observations of V. invictae in Solenopsis richteri and **S.** macdonaghi. No infections were detected in 12 non-Solenopsis genera and 235 non-ant arthropods in 10 orders, 43 families, and 80 species (Briano et al. 2002, Porter et al. 2005).

Laboratory colonies of the tropical fire ant, Solenopsis geminata, and the southern fire ant, Solenopsis xyloni, which are found in the U. S., were inoculated with brood from *V.* invictae infected colonies (Oi et al. 2005). No infections were detected microscopically or by PCR (Valles et al. 2004) in the S. geminata (n=4) and S. xyloni (n=5) colonies. In contrast, 3 of 5 inoculated S. invicta colonies had *V.* invictae infections. All control colonies (n=14) remained uninfected.

The importance of transovarial transmission of *V.* invictae is uncertain, where there were only 2 infected queens, out of 12, collected from infected colonies. In addition, there was a low prevalence of infected eggs, where vegetative stages of *V.* invictae was found in "some" eggs out of 515 (Briano and Williams 2002). In preliminary studies on the prevalence of transovarial transmission of *V.* invictae, 5 physogastric queens from *V.* invictae-infected fire ant colonies collected near San Javier, Argentina were all uninfected based on PCR detection. In addition, larvae reared from eggs laid by 10 queens from *V.* invictae-infected colonies collected in Argentina were uninfected. Lastly, in groups of approximately 100-500 eggs (n=6) from 6 queens of laboratory-infected S. invicta colonies, *V.* invictae was not detected. Thus, transovarial transmission of *V.* invictae was not evident. However, infection of queens in the latter studies was not confirmed and hence results are not conclusive.

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Chuck Strong (USDA-ARS CMAVE) provided assistance with PCR determinations of *V. invictae* in some samples.

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Characteristics of *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) Population Spread in Louisiana

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Knowing the rate of spread of organisms is important for predicting future movements into new areas, based on characteristics of previous dispersal patterns. Expansion rates of animal populations generally follow either a constant or accelerating pattern over time when the square root of the area occupied by the species is plotted against time. Species that are characterized as having a stratified (jump) dispersal pattern tend to display an initial latent period where spread rates are low. Over time, a proportion of individuals in these populations disperse far ahead of the advancing surface wave and nascent far-flung colonies are established. These colonies spread in a similar fashion to the parent colony, and an accelerating (quadratic) expansion pattern results. We studied the expansion of two south Louisiana P. tricuspis populations from 1999 to 2005. By disturbing red imported fire ant *(S.* invicta) mounds in four cardinal directions (north, south, east and west) from each release location and determining presence/absence of P. tricuspis, we were able to approximate the yearly limits of population spread of this species. It was found that the square root of the area occupied by P. tricuspis over time is described by a quadratic function, indicating that expansion of P. tricuspis populations in Louisiana follow **a** stratified dispersal pattern, i.e. local (neighborhood) diffusion and long-distance dispersers. Our P. tricupis populations exhibit a latent phase whereby dispersal is slow for several years, followed by accelerating spread to an asymptotic rate of approximately 16-27 km/year, depending on direction from the release areas. Spread patterns were found to be anisotropic with respect to the position of the release point: slower to the south and faster to the north. A putative explanation for this pattern is that flies are being transported longer distances to the north as a consequence of sea breeze wind patterns that flow from south to north along the Gulf coast during the summer months.

The Red Imported Fire Ant, *Solenopsis Invicta* in Mainland China

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The red imported fire ant (RIFA), *Solenopsis invicta* Buren was first discovered in China in Wuchuan County of Guangdong province in September of 2004 (Zeng et al., 2005). Subsequently, it was also found in other districts of Guangdong, Guangxi, Hunan and Fujian province. In total, 16 districts were sporadically infested by the end of 2005 with the largest infestation occurring in Guangdong province. The RIFA had a serious impact on human health, agriculture and biodiversity in all the infested areas. The discovery of the RIFA and level of damage it caused sent shock waves through urban and rural communities alike. In January of 2005, the Ministry of Agriculture of China (MAC) declared the RIFA as a quarantine pest and called for a campaign to isolate and eradicate it as soon as possible.

Insecticides have been the primary method of control of RIFA in mainland China. The first control method used was individual mound drenches (IMD). The insecticide used was mainly lambda-cyhalothrin. The top of the mound was dug out after a trench was made around the mound and diluted insecticide was poured into it. Most field treatments were estimated to cause 80-95% colony mortality. The credibility of this estimate is suspect; however, since the efficacy of this treatment method has not been evaluated. Since IMD was rarely effective in reducing the overall RIFA population, the major control means shifted from IMD to baiting. Some of the major ingredients for the baits were the same as those used in the United States. Most of the baits were applied around or on the mounds by a table spoon since no bait broadcaster was available. The available baits provided a control efficacy of 75% to 100% depending on the type of the bait and how and when it was used. Nursery stocks and potted plants were treated with aquatic lambda-cyhalothrin when necessary.

Some major progress was made in both the control and describing the ecology of the RIFA in 2005. First, damage and colony density of RIFA were greatly reduced which allowed people in the infested areas to have a normal life and agricultural activity. Second, a new bait, called NBPS, was developed and in early trials exhibited excellent control. Its LD_{50} to the rat is 2000 mg/kg and therefore has a low mammalian toxicity. The efficacy of NBPS bait was significantly greater than either fipronil or fenoxycarb after 8 weeks in a field test. The mortality of colonies treated with the bait was 83.5, 93.9 and 100% after 1, 2 and 3 weeks, respectively, when temperature was over 25°C and there was no rain within 48h of application. Third, eradication efforts were tried in two infested sites with encouraging results. Four hundred twenty eight visible mounds on 3.3 hectares (□22°19.62′, E110°16.08′) were killed within 3 weeks after application of 40 kg of NBPS bait. The treated area remained free of RIFA mounds after nine months. In a second test, 3272 colonies on more than 380 hectares (N 22°80′, E108°19′) were also killed within four months by application of 76.5 kg of NBPS bait. However, nine mounds were found on the untreated perimeter of the site where the bait was not applied in early 2006. Fourth, some observations were made on the ecology and biology of RIFA. For instance, although mounds were distributed across varied habitats, the majority occurred in sunny, weedy and areas near water, but few occurred in forested areas. The RIFA colonies found in most of the infested areas were polygyne forms and their life history was similar to that reported in the southeastern USA. Finally, some natural enemies were found that have an adverse impact on

RIFA populations. For example, spiny-weaver ant, *Polyrhachis dives* workers were observed to fight and defeat RIFA workers and its colonies successfully nested near RIFA mounds. The fungus *Beauveria bassiana* was pathogenic to the RIFA in laboratory tests at Zhongshang University. A recent investigation showed that the average speed of expansion by the fire ant in Guangdong was only 187.9 m/year (Xu et al., 2006); however, the original infestation date was not known.

Overall, the total RIFA infested area in mainland China was small, and its natural dispersal speed in the country might not be as fast as that reported in the USA. Also, the central and local governments of China are very concerned about the presence of RIFA in the country. These things, taken together, favor the control of *S. invicta* in China. However, the challenges we face are obvious since application of insecticide is the only suppression method we have, and factors that limit control efficiency are difficult to overcome at present. In addition, our monitoring technology isn't adequate, and our quarantine rules are not yet standardized. We therefore, still have a long way to go in terms of developing either sound RIFA integrated control or an eradication program.

Acknowledgement:

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The Effects of Fire Ants on an Old Field Arthropod Community

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Abstract

The red imported fire ant, Solenopsis invicta, is a highly abundant, voracious generalist predator. In a field study we quantified the effect of fire ants on arthropod communities in old-field settings. Thirty 2 X 3 m plots were partitioned and commercially available ant-specific insecticide bait was applied to half the plots. Each plot was visually surveyed weekly. Arthropods were identified and their abundance recorded. We found strong evidence that fire ants affect the arthropod community. For instance, in areas of ant suppression there was a dramatic increase in several of the 25 arthropod groups surveyed (Araneae, Chrysopidae, Cicadellidae, Coccinellidae, Diptera, Reduviidae, Hymenoptera). In contrast, areas with fire ants had a significant increase in 1 of the 25 groups (Cercopidae). This increase in spittlebug nymphs was not expected. We hypothesize spittlebug predators such as spiders are suppressed by fire ants, while the mass produced by nymphs deters ant predation.

Results

The presence of S. invicta had a significant negative impact on 28% of arthropod groups surveyed. However spittlebug nymphs appear to benefit from the presence of fire ants. In control plots (high fire ant) spittlebug nymphs were significantly more abundant compared to Amdro treated (low fire ant) plots (Figure 1). This trend was consistent throughout all sampling dates. We hypothesize that the presence of fire ants decreases the abundance of spittlebug predators such as spiders (Figure 2) whereas ants tend to ignore the spittle mass.

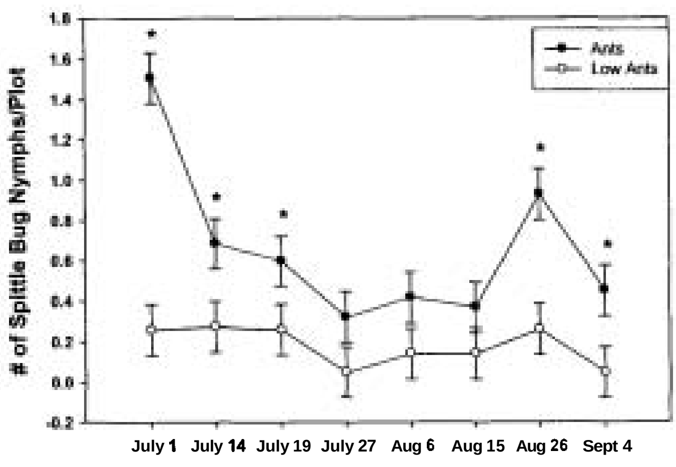

Figure 1 Mean plot densities of spittlebugs comparing plots with suppressed ants (Low Ants) and control plots (Ants). Ant presence significantly and consistently increased the abundance of spittlebug nymphs.

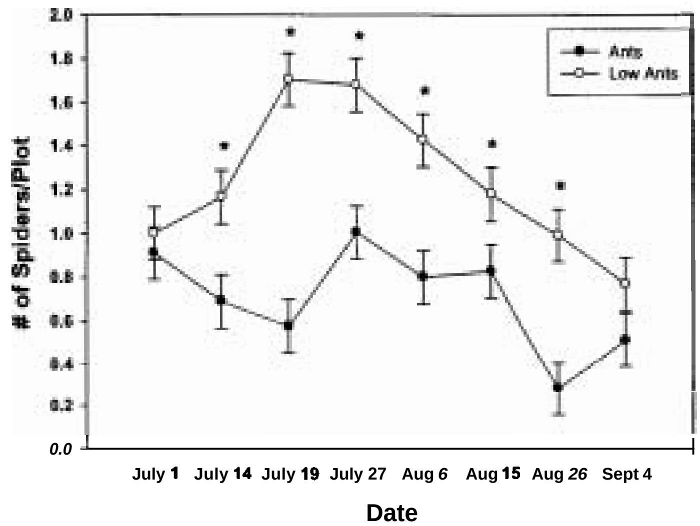

Figure 2 Mean densities of spiders in plots with suppressed ants (Low Ants) compared to control plots (Ants). Spider abundance was significantly and consistently decreased in plots with ants.

Effect of Hurricane Katrina Flooding on Ants of Orleans and St. Bernard Parishes, Louisiana

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Hurricane Katrina struck the Gulf coast on August 29,2005, with some areas receiving 30 – 40 cm of rain and storm surge reaching heights of over 9 m. A combination of wind, rainfall and storm surge caused breaks in the levees separating New Orleans from the surrounding lakes and canals, leaving some areas under 6 m of water, with at least 80% of the city being flooded and some neighborhoods remaining under water for several weeks. In addition to the devastation caused to residents and their homes, flooding from storm surge and levee breaches had major environmental consequences.

In October 2005 we began an ongoing study of the effects of flooding on ant populations in Orleans and St. Bernard Parishes, Louisiana. We compared pre-and post-Katrina ant species compositions at sites where we had previously sampled. We also mapped the distribution of ant species in other areas impacted by Hurricane Katrina. As of March 2006, we had collected 10 of the 20+ species previously found in New Orleans City Park, a moderately flooded area that received an average of approximately 2 m of water. Ant populations in flooded areas throughout Orleans and St. Bernard remained drastically reduced. Parts of St. Bernard Parish and New Orleans' Lower 9^{th} Ward, Gentilly, Lakeview, and New Orleans East neighborhoods were free of the red imported fire ant and contained few or no other ant species ants.

Fire ants are known to form clusters that allow them to float on the surface of water. Because large parts of the New Orleans area were fire ant-free after Hurricane Katrina we conducted lab experiments to determine how long fire ants can survive in water with salt concentrations ranging from 0% to 3.5% (the concentration found in sea water). Ants immersed in water with more than 3.5% salt lost their ability to form the ball-like clusters that allow them to survive flooding. Within 30 min., fire ants were trapped under the surface of the salt water and sank. Decreasing the concentration of salt allowed the ants to survive longer. In salt-free water, fire ants were only able to survive for about six days before the balls began to break apart and the ants sank. In addition to time and salt concentration, the speed of inundation may also be a factor in ant survival

Based on the results of our field surveys we developed a plan to delay re-introduction and establishment of fire ants in areas where they had been eliminated. This plan consists of 1) an education campaign training residents to recognize and bait for fire ants, 2) large-scale baiting in neighborhoods and green spaces, and 3) a long-term maintenance phase to prevent a monoculture of fire ants and allow beneficial insects to become established.

Residential Red Imported Fire Ant treatments in Orange County, CA. A partnership with private pest control companies

Charlie Cassidy, Vector Control Inspector III, James Green, Vector Control Inspector II and Michael **Hearst**, Public Information Officer

Orange County Vector Control District, 13001 Garden Grove Blvd., Garden Grove, CA 92843

History

The Orange County Vector Control District (OCVCD) has been the primary agency conducting mosquito abatement in Orange County, California since 1947. OCVCD is one of the few California vector control districts that places rodenticides to control rats. Along with mosquitoes and rat control, The District has one of the largest Red Imported Fire Ant control programs of any California vector control district. In addition, OCVCD provides public education on many other vectors and pests and has done control operations on other vectors such as Africanized honey bees and sculptured scorpions when needed.

Red Imported Fire Ants (RIFA) *Solenopsis invicta* Buren were discovered in nursery stock sent to Nevada in fall 1998 from Orange County, CA. The California Department of Food and Agriculture (CDFA) immediately began delimiting the infestation. Major infestations were discovered in north and south Orange County. As a result, quarantine was implemented on nursery stock and soil movement for all of Orange County.

The California Department of Food and Agriculture contracted with local agencies to work towards eradication of RIFA in Southern California. The Orange County Agricultural Commissioner received the contract to treat and survey for RIFA in Orange County. The Agricultural Commissioner's office subcontracted with The Orange County Vector Control District (OCVCD) to do treatment, survey and conduct public outreach for RIFA. The Orange County Fire Ant Authority (OCFAA) was established as a sub-agency within OCVCD and started work in February 2000.

The program ran for four years, from February 2000 to February 2004. OCFAA conducted treatment and survey in residential areas, parks, schools and businesses. Over 29,000 sites were treated in the four year period. More that 15,000 of these completed the treatment cycle of four treatments at 3 to 4 month intervals. Post treatment monitoring began 3 months after the final treatment. Post treatment surveys showed less than 5% of these sites remained positive for RIFA.

OCFAA's state funding was ended in September 2003 due to massive state budget deficits. The County of Orange funded the program for the next four months while other funding sources were investigated. Unfortunately, with no funding available the OCFAA ended operation in February 2004.

OCVCD Program

The Orange County Vector Control District was preparing for West Nile Virus to enter California in spring 2004. The District needed to secure stable funding to increase mosquito abatement and surveillance activities to better protect the residents of Orange County from arboviral diseases. Red Imported Fire Ants were still a problem in many communities and no organized control was being done.

The District's Board of Trustees voted to include RIFA as a "vector" the district would actively control. Property owners within Orange County were asked to vote on a benefit assessment to fund these efforts. This would be an additional fee added to their property taxes to control mosquitoes more effectively and restart a RIFA program. The measure passed in the summer of 2004.

A new Red Imported Fire Ant program was kicked off in October 2004. The new program is incorporated into the operations of OCVCD. Three full time vector control inspectors coordinate large area treatments and surveys. Seasonal staff are hired for 9 month periods to complete treatment and survey in parks, schools greenbelts, public medians and commercial businesses. In addition, twenty full time inspectors working on mosquito and rat abatement inspect residential properties when complaints come in from the public. Samples are taken and species are verified by OCVCD laboratory personnel. Individual mounds are treated with Amdro® Pro (0.73% Hydramethylnon) at a rate of 2-5 tablespoons per mound. Amdro® Pro is then broadcast over the remaining property and adjoining properties at a rate of 1 – 1.5 lb. / acre.

Residential site records are completed with contact information for neighboring properties and treatment information. (Figure 1) Property owners are instructed to call OCVCD if new mounds arise after a 4 week period. The partnership between local pest control companies is explained to homeowners. The companies will return at approximately three month intervals to treat with an insect growth regulator, Distance® Fire Ant Bait (0.50% pyriproxyfen) for the second and third treatments. After the third treatment has been completed, post treatment monitoring will be done by OCVCD staff to determine if the RIFA are still present.

Partnership

Work orders are sent to The Pest Control Operators of California (PCOC) in Sacramento California the month before the second treatment is to occur. Work orders are then distributed to 12 participating local pest control companies that are members of PCOC. This is done to avoid any favoritism or conflict of interest by OVCVD. Companies receive a pre-agreed payment for each site relating to the size and number of properties treated. Small properties under 1/5 of an acre receive a $10.00 each. Medium properties from 1/5 to 1/3 acre receive a $12.50 each. Large properties over 1/3 acre in size receive $15.00 each. In addition, each site will receive a minimum of $50.00 per work order. Sites generally contain four or five properties per work order.

Pest control companies will contact the property owners after the three month interval to set up a treatment time. The PCOs broadcast Distance® Fire Ant Bait (0.50% pyriproxyfen) at a maximum label rate of 1.5 lb/acre. Treatments are recorded on the work order; the work orders are copied and returned to OCVCD with invoices for verification and payment. Currently the partnership of OCVCD and PCOC companies are treating over 500 residential sites or close to 1900 single family homes. As neighborhood surveys increase in the coming year we project this number of sites to increase by two to three fold. Outreach activities to raise public awareness will also widen the visibility of the program and more reports will come in from residential property owners.

Infestation Pattern

Residential treatments have followed the pattern of large area infestations in Orange County, California. The heavily infested areas remain in the North and South Orange County cities with little activity in central Orange County. (Figure 2) The northern infestation has spread to the east with newer development in the northeastern cities. There has been a reduction in the number of active RIFA sites in South Orange County where the majority of active sites occurred from **2000** to **2003.** (Figure **3)**

Benefits

The current residential treatment program has been of mutual benefit to both Orange County Vector Control and local pest control operators belonging to PCOC. OCVCD staff workload is reduced, freeing them to do large area treatments and surveying for new areas to find unreported colonies. The program allows OCVCD staff to train the **PCOs** and insures treatment protocols are followed. Partners in the pest control industry receive training in proven RIFA control methods for their staff. The program gives companies the opportunity to offer additional services to homeowners. Finally, with proper application of appropriate control chemicals there is substantially less chemical used by homeowners that can end up in our local environment. These aspects benefit all the residents of Orange County. The program helps to open the door for future cooperative ventures that can help reduce costs in controlling vectors and invasive agricultural pests.

Figure 1

Fire Ant Work Order (Residential)

Work Order No. 1569

MAIN CONTACT

Name	TOM MILLER		
Address	1234 ELM ST		
City, State	Santa Ana		
Phone (H)	(714) 555-5555	Zip Code	92702-
Phone (W)		Extension	
Phone (C)			
# of Dogs	1		
Aggressive	N	Page/Grid	828D3

Service Request Number			[illegible]
Date Reported to Vector Control			1/1/2006
Date Work Order Sent to PCOC			4/1/2006
Size and No. of Properties	S = 2	M = 3	L = 0
$ Totals	$20.00	$37.50	$0.00
Amount to be Billed			$57.50

Sample Taken? Y

Confirmed Positive? Y

		Date Treated	Chemical Used	Amount	Unit	Number of Mounds	Technician Name	Minutes Spent
Date of Initial Assessment/Treatment		1/3/2006	Amdro	IS	Oz.	1 - 10	Charlie Cassidy	45
Month for 2nd Treatment	April							0
Month for 3rd Treatment	July							0
RATE a APPLICATION FOR DRY BAIT (BROADCAST TREATMENT) IS 1.5 LBS. PER ACRE								

Properties to be Treated	1st FY	1st BY	2nd FY	2nd BY	3rd FY	3rd BY	Comments
1234 ELM ST	✔	✔					
1236 ELM ST	✔	✔					DOG IN B/Y
1238 ELM ST	✔	✔					FRED HAYES 714-555-1000
1232 ELM ST	✔	✔					MARTHA MUFFIN 714-555-1001
1230 ELM ST	✔	✔					

ADDITIONAL PROPERTIES LISTED ON BACK

Orange County Vector Control District
[illegible] Garden Grove Boulevard Garden Grove, CA [illegible]
[illegible]
RIFA - Charlie Cassidy Ext. [illegible] [illegible] Ext. [illegible]

FORM NO. [illegible]

Figure 2

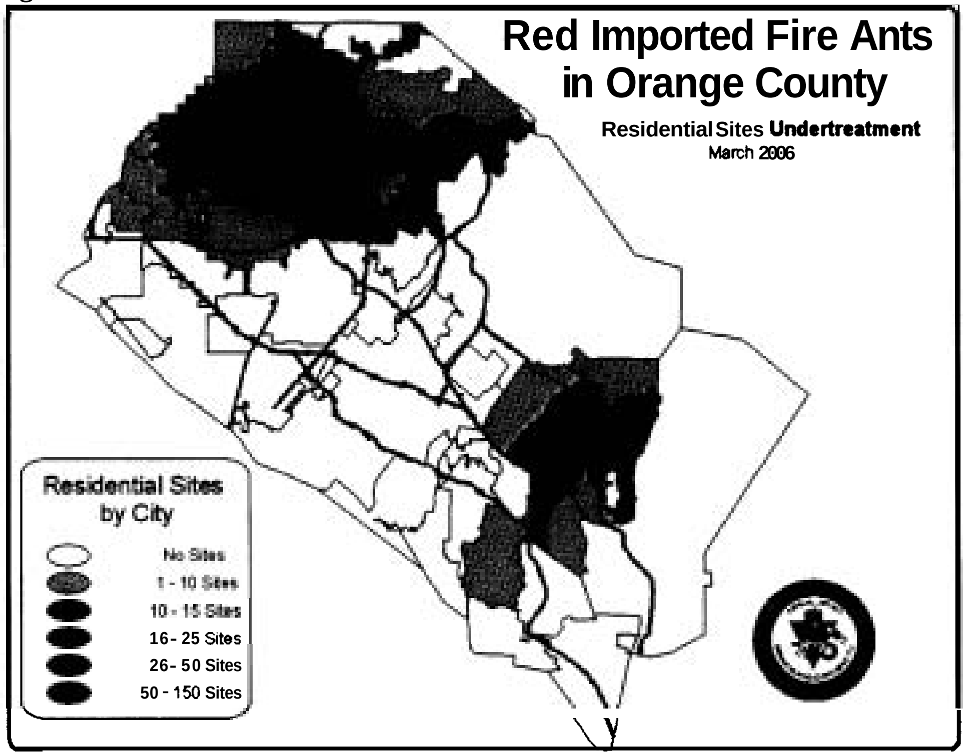
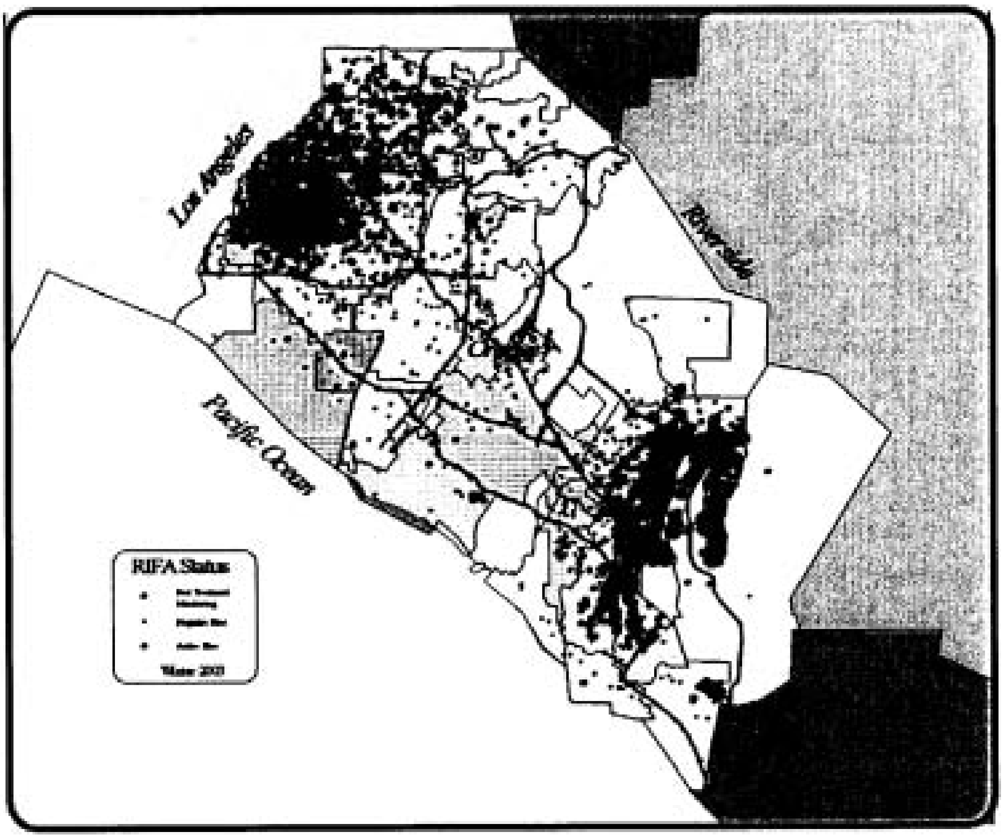


Figure 3. 2003 RIFA Sites



Surveillance Methodologies Used within Australia. Various Methods Including Visual Surveillance and Extraordinary Detections; Above Ground and in Ground Lures.

Cara McNicol

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This paper will discuss the surveillance methods used as part of the Australian National Red Imported Fire Ant Eradication Program.

Program Overview:

Firstly an overview of the Australian program is provided to give some background with the level of the infestation and the size of the program currently being undertaken in Australia. The eradication program commenced in 2001, with an original plan of **3** years of treatment, with 2 years following of surveillance to assess the efficacy of the treatment applications. This program was funded from 2001 through until 2006. Subsequent significant detections in the early years of the program resulted in an extension to the program for a further year of treatment and surveillance, with the program currently funded through until June 2007. This is a $175 million dollar program with the funding shared between the Commonwealth and State governments.

The program is currently in its fifth year and covers the following area for each application respectively; Surveillance: 62,250 hectares across 150,850 sites and Treatment: 6,200 hectares across 16,950 sites. The program is located primarily in the south east corner of the state of Queensland on the east coast and employs in this fifth year of the program; 250 field staff to perform active surveillance activities and 50 field staff undertaking the treatment applications on a full time basis. There are approximately a further 100 support staff employed to maintain the program at this size.

Surveillance Methods

To conduct the surveillance; a range of active and passive surveillance methods are employed. The active surveillance methods used on the program are visual surveillance, in ground traps, pit fall traps and above ground lures. The passive surveillance methods used include community reports and volunteer watch groups.

Visual Surveillance

The most common method of surveillance used operationally on the program is visual surveillance. This is conducted by field staff lining up standing side by side approximately two meters apart. This is termed an "emu parade" method as this is behaviour displayed by the native Australian bird; the emu, whereby the bird will pick at the ground with its beak as it walks along in search of food.

Visual surveillance is extremely labour intensive with currently 250 staff employed on a full time basis to perform this task. There is very minimal equipment required to conduct visual surveillance, staff being provided with a 1 ½ - 2 meter stick to prod the ground when testing for the presence of suspicious ants. The program requires 100% of vegetated areas across all surveillance sites to be inspected. Staff can effectively conduct surveillance at an average rate of 1.25 hectares per person per day. To enable safe access to a diverse range of sites and land uses, staff are provided specialist training and induction to enter commercial and industrial construction sites, road and rail corridors, riverbanks and low lying swamp areas.

Challenges of visual surveillance

The need for staff to physically walk across areas to visually inspect for the presence or absence of fire ants presents some challenges. These are particularly related to heavily vegetated areas where it is difficult for staff to safely walk through an area and to see the ground due to heavy vegetation cover. These present considerable safety issues in particular with the presence of snakes in bush areas and throughout residential areas the largest safety concern for staff are dangerous and aggressive dogs. In addition to this, maintaining staff concentration and morale is a challenge and significant time and effort is invested to promote staff concentration and morale.

Sensitivity of visual surveillance

To ensure that staff are effectively conducting visual surveillance, surveillance sensitivity is measured, using imitation fire ant mounds which has over the period of the program returned a range of results between 65 % to 85 %. New quality assurance methods being used have very realistic fire ant mounds and have improved the sensitivity to consistently return results across the program of 84%. Quality assurance methods used also include placement of business cards under pot plants and behind sheds and conducting interviews of the residents as to staff performance.

Extraordinary fire ant detections

Despite the challenges, field staff conducting visual surveillance have detected many extraordinary fire ant finds. Staff have detected the very early beginnings of a nest amongst exposed soil, identified only by the different colour of a patch of soil which has been the subsoil that has been brought to the surface by the ants in the construction of a new nest. Particularly with the success of the treatment program and low levels of fire ant populations, staff have detected extremely small and new nests.

Vegetation clearing

To overcome the difficulties associated with accessing heavily vegetated sites and to ensure that 100% of sites within the defined surveillance zones are inspected for the presence and/or absence of fire ants, the vegetation is cleared to enable inspections. In ground traps are used in heavily vegetated areas, where without clearing staff could not penetrate to access.

Specialist staff are trained and outfitted with brush cutters and contractors are engaged to use excavators for thick wooded areas to clear vegetation. Tracks are cut which enable teams to penetrate through the vegetation to place in ground traps along a grid.

In ground traps

In ground traps are placed in grids of 10 meters x 10 meters as a standard square grid. Where there is low likelihood of fire ants being present, a 30 meter x 10 meter grid may be used. Areas identified for vegetation to be cleared the grid lines are predetermined using maps with aerial imagery to identify the most suitable access for the machinery and the particular land features that need to be navigated around, ie rivers, dams, etc. Where there are land features that cannot be crossed such as a dam / swamp area, along road and rail corridors traps are placed along the perimeter of these features.

The trap is a large plastic centrifuge vial with holes drilled in the side and a pot marker used to identify the location in the ground. A dry dog biscuit "Good-0" is used as a bait, with the beef flavour having been found to be more effective than the chicken flavour. The bait is suspended using either a wire or a mesh guaze with a preservative fluid put in the trap to preserve caught ant samples. Propylene glycol has been the most effective preservative fluid available. Traps are placed in the ground with a modified crowbar which is used to puncture the ground at the right height and allows staff to walk along and place traps in the ground very quickly. Traps are left in the ground and recovered between 3 and 5 days after being placed. In ground traps are relatively quick to place and recover with a field team (13 staff) able to place between 500 – 600 traps per day and recover between 600 – 700 traps per day. Field teams recover the entire trap and return the trap as a whole to diagnostic staff. This makes the in ground traps quick to use in the field but are very slow for diagnostic staff due to the samples often being mixed and dirty with other contaminants collecting in the trap including non suspect ants, soil and some bait residue. In ground traps also need to be maintained whilst in the ground when there are rain events. If not adequately maintained shortly after rain, the traps may flood and samples may be potentially lost and/or the preservative fluid is lost and replaced with rain water, resulting in samples becoming mouldy and extremely difficult to identify.

Above ground lures

Above ground lures are used in moderately vegetated areas, these are areas where staff can physically walk through however visual surveillance throughout this vegetation would not be effective as the ground is not visible. Above ground lures are used in conjunction with both visual surveillance and in ground traps depending on the vegetation on any particular site.

Above ground lures are a small square vial with holes drilled in the sides to permit ant entry / exit and to secure to the ground with a pot marker – also used to identify the location of the lure. A piece of processed meat, referred to as a "cheerio" or hotdog sausage is placed inside the vial. Long grass or other vegetation is separated to ensure the lures are placed in contact with the ground. Lures are placed early in the day when the temperature is cooler and the humidity is lower. The lures are then checked throughout the day for the presence / absence of suspicious ants only. Individual ant samples are collected from the bait and submitted as a clean sample. The use of above ground lures is much slower in its field application but has the benefit of quicker diagnostics due to clean "suspect only" samples being submitted. Lures are only left in the field for a few hours, whilst the staff are completing visual surveillance across the remainder of the site. Above ground lures have been found to be most effective with high humidity.

Pit fall traps

Pit fall traps are only used for scientific experiments and not as an operational method in the achievement of the surveillance zones required each year. Pit fall traps are an open centrifuge vials which have a preservative fluid similar to an in ground trap, but do not have any bait or attractant in them. Pit fall traps are only left out for a short period and used across very small grids. Pit fall traps rely on the ants falling into the trap while out foraging and then being preserved in the fluid in the trap until collected. Similar to in ground traps, the entire pitfall trap is collected and returned for identification, which can be quite time consuming and labour intensive depending on the content of the traps and the intensive grids used for pitfall traps.

Smart Science

A clever tool has been developed which targets and prioritises the areas to conduct surveillance as it is extremely slow and costly to conduct visual surveillance across large areas ie. 62,000 hectares. Scientific modelling resulted in the development of the Australian Fire Ant Habitat Model. This model identifies the areas to look for fire ants based upon the probability and likelihood of fire ant infestation and population establishment. The habitat model uses factors such as layers of disturbance through development and topography and uses landsat imagery. The model was originally developed in 2002 using data across the original core infested area, with half the area used as the training and development area and the other half of the area was used to test the model. This original model using 2002 data points of infestation and 2001 imagery returned an estimated success rate of probable locations for fire ant infestation of 98.3%. The model identified a gradient scale of areas that would most likely support fire ant infestations in 10% gradient increments. To maintain the 98.3% success rate, the interpretation of the model requires that the 500 meters surrounding any infested site is inspected.

The model was recently refined in 2005 and tested with a more recent data set. This refined model resulted in a significant reduction and therefore cost savings of 60% of the area required for inspection but which predicted the detection of 98.8% of colonies. If 50% of the area was inspected, the success rate predicted for detection of fire ant infestation increased to 99.3%. This still required that the 500 meters surrounding each infestation is inspected to maintain those success rates. The model has also been tested against fire ant detections made by members of the public and returned the same success rates. Furthermore the habitat model has been validated against external data sets, including colony position data from other countries with the same high success rates of 99%. The success of the model in a different country with a different environment supports its use as a successful tool to efficiently and effectively guide surveillance efforts in areas of low infestation.

Program Statistics for Active Surveillance Efforts

Since the commencement of the program in 2001, there has been considerable active surveillance effort undertaken, covering inspection of a total of 224, 198 hectares across 291,960 sites, which required 664,325 visits to successfully access and conduct surveillance over these sites. A total of 3,164 nests have been detected through the use of visual surveillance, placement of 21,000 above ground lures and 110,000 in ground traps. Furthermore the task is not yet complete, with considerable ongoing active surveillance efforts required to ensure detection of the last fire ant nest in Australia.

While conducting surveillance for fire ant, a number of incursions of other ant species have been detected, including Argentine ant, Crazy ant and Tropical fire ant. Other items detected through active surveillance efforts include recovery of stolen and/or lost property. There have even been some gruesome detection's made by unsuspecting fire ant staff including animal and human bodies and remains being found. Staff working across such large areas and so many sites has also resulted in many Good Samaritan deeds being done, including recovering lost dogs, assisting members of the public with vehicle breakdowns and reporting and assisting with extinguishing wildfires. This program is more than just looking for and eradicating ants.

The success of the Australian Fire Ant Eradication Program

The program to date has been extremely successful with secure funding which has enabled effective treatment applications, a high level of public support and public cooperation permitting access for staff to inspect 100% of private and difficult sites and having a high number of public reporting of suspicious ant samples.

The most difficult challenge which faces the Australian eradication program is the same that is faced by any eradication program – to find the last one. The treatment application is the easy part of the program, as we know it is highly effective, but finding that last Red Imported Fire Ant……..is the challenge.

extension: Taking the Sting out of Imported Fire Ants

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The authors of this paper are co-leaders of the Imported Fire Ant Management Community of Practice. This Community is charged with developing information on imported fire ants and their management, that can repackaged to fit various electronic delivery platforms, and be available 24171365 to our Community of Interest.

This Community of Practice plans to provide information on imported fire ants in English and in Spanish. In 2006, we will develop frequently asked questions about fire ants, an interactive decision module to provide customized fire ant management advice, and basic information on managing imported fire ants in urban areas and in cattle production systems. In future years, additional decision modules and learning modules will be developed, and the amount of basic information will be expanded. The Imported Fire Ant extension site will be formally launched in March 2007. More information on this community of Practice can be viewed at: *http://cop.extension.org/wiki/Imported_Fire_Ants_Community*

We encourage you to join the Imported Fire Ant Community of Practice.

Movement Controls used to prevent Red Imported Fire Ant spread in Australia

Jason Haffenden, Manager Program Compliance

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Background

Red Imported Fire Ant (Solenopsis invicta) was identified in Brisbane in early 2001. Subsequent surveillance resulted in the detection of two main loci and a number of smaller outlying infestations. The National Fire Ant Eradication Plan was developed in response to this detection and implemented later in 2001.

The Queensland Department of Primary Industries & Fisheries (QDPI&F) is the lead agency in the formation and delivery of the National Red Imported Fire Ant Eradication Program currently being undertaken in South East Queensland. This program is funded via a national cost share funding agreement between all states, territories and the Australian government. The Program currently involves an area of approximately 70 000 hectares, of which 62 000 hectares is undergoing Surveillance and the remainder eradication treatments.

As part of the eradication plan, Fire Ant Restricted Areas were declared to define the area where Red Imported Fire Ant Movement Controls have been applied. Movement controls have been implemented to prevent the human assisted spread of Red Imported Fire Ant (RIFA) and contain the infestation to areas of the eradication program. A risk based assessment is used to determine Restricted Area boundaries. Pending the size, number and apparent age of the infestation found, the Fire Ant Restricted Area boundary can vary from 500 metres to two (2) kilometres. The entire area undergoing eradication activities has been declared a Fire Ant Restricted Area.

Movement Controls

In development of RIFA Movement Controls, a stance of acceptable risk rather than nil risk was taken given the potential list of possible vectors and the areas where these controls needed to be implemented. RIFA movement controls affect a wide range of businesses, with most being previously largely unregulated or exposed to limitation placed on the conduct of their business activities. However, given the significance of this pest and the seriousness of the response to its incursion, stringent movement controls were implemented.

In establishing RIFA movement controls, QDPI&F used existing United States Department of Agriculture (USDA) Red Imported Fire Ant Movement Controls as a basis and augmented these with input from key stakeholders including relevant authorities from funding partners and potentially affected industry groups.

Queensland's RIFA Movement Controls have been legislated under the Plant Protection Regulation *2002* subordinate legislation to the Plant Protection Act 1989, with fines of up to $375 000 able to be imposed. However a focus of extension rather than coercion has been used to inform the wider community of Movement Control requirements.

RIFA Movement Controls are imposed on all properties within the Fire Ant Restricted Area and are predicated on allowing controlled movement of High Risk Items within and from the Fire Ant Restricted Area. High Risk Items are defined as landscaping materials, potted plants, mulch/hay and bulk soil or machines used to move bulk soil. Additional items are evaluated on a case by case basis as to their potential to harbour RIFA. If deemed as potential carriers, they also fall under the requirements of RIFA movement controls.

All movements of possible host items require control measures. These control measures vary based on: the High Risk Item; its origin; how it has been produced or stored, and its intended destination. Movements of High Risk Items are subject to treatment and or inspection prior to movement unless appropriate, approved hygiene requirements are implemented. Movement of High Risk Items outside of the Fire Ant Restricted Area requires a higher degree of control measures to be undertaken prior to movement. Movements of High Risk Items are required to be accompanied by certification endorsed by the supplier as complying with RIFA Movement Controls.

Commercial operators working within Fire Ant Restricted Area are required to maintain an Approved Risk Management Plan which outlines operators' requirements in relation to preventing further spread of RIFA. Factors specified in an Approved Risk Management Plan include: purchasing requirements; movements on and off site within the Fire Ant Restricted Area; storage of Restricted Items; on site monitoring for RIFA, and staff training. Businesses on an Approved Risk Management Plan are categorised as product suppliers and or service providers.

Product suppliers, i.e. businesses such as nurseries that store or trade High Risk Items within the Fire Ant Restricted Area, are required to store their product in a way that prevents terrestrial infestation or apply mandatory treatment prior to movement. Businesses are required to maintain various records to substantiate completion of actions. Businesses are also required to supply appropriate movement certification with every consignment.

Service Suppliers are businesses that supply a service, such as builders and earthmovers. These businesses are required to ensure that machinery used to disturb earth in the Fire Ant Restricted Area is cleaned prior to movement and that no soil extracted from the Fire Ant Restricted Area leaves the Fire Ant Restricted Area. Further, there is a requirement that a pre-disturbance inspection of the site is completed by an Approved Person where there is to be disturbance of greater than 1 cubic metre of soil. Subsequent inspections on a 28-day basis are undertaken by the principle contractor/owner until work is complete.

In developing an Approved Risk Management Plan, Inspectors examine the business' existing processes or procedures to evaluate how these may already be reducing the risk of spreading RIFA. An example of this is compost manufacturers. As part of the composting process temperatures in excess of 65 ^{o}C are reached and therefore deemed as sufficient to destroy life stages of RIFA. Requirements of verification of temperatures and post production storage and handling are documented thus fulfilling movement control requirements.

Approved Risk Management Plan holders are subject to formal audit by Inspectors. In addition to formal Audit random audit of weekend markets, new housing estates and trade shows occurs to verify compliance with Fire Ant Movement Controls.

Communication is the key to gaining compliance with Fire Ant Movement Controls. Having members of the community enquire of their contractors or suppliers, "What are you doing to prevent the spread of Fire Ants?" In addition, visual reminders such as billboards, television and radio advertisements, businesses displaying signage and a regulatory presence all contribute to the overall compliance, collective effort and effectiveness of the eradication program.

A Regulatory Perspective on Imported Fire Ant Research

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The Annual Imported Fire Ant Research Conference represents an opportunity to learn about advances in many facets associated with fire ants such as behavior, management, and public education. Attendees at Fire Ant Conferences come from many different backgrounds including academic, industry, and government. The purpose of this presentation is to highlight research that is of particular interest to us who regulate the movement of commodities that could be infested with fire ants.

The purpose of the Federal Imported Fire Ant (IFA) Quarantine is to prevent the artificial spread of IFA from the quarantine area which consists of all or part of 13 States and the Commonwealth of Puerto Rico. Regulations for the IFA Quarantine are in 7 CFR 301.81 (Code of Federal Regulations). Each fiscal year Congress appropriates funds that APHIS provides to 1) states fully or partially infested with IFA to assure regulatory compliance within those states; and 2) states uninfested or only partially infested to survey for IFA and determine areas the should be added to the quarantine.

Administration of the IFA Quarantine is by staff at the APHIS Headquarters in Riverdale, MD, at Regional Offices in Raleigh, NC and Ft. Collins, CO, and by the PPQ State Plant Health Directors in most every state. An integral part of the Federal Quarantine is the Soil Inhabiting Pests Section, also known as the Fire Ant Lab, in Gulfport, MS. At this facility quarantine and survey methods are developed for regulated commodities. While this lab has a long history of producing effective treatment technologies for the IFA Quarantine, APHIS is also interested other research that could be applied to the activities of the Federal Quarantine.

Developing IFA survey techniques is one area where research can assist APHIS. There is a particular need for a rapid field identification technique that can accurately determine if an individual ant is either Solenposis invicta, S. richteri, or their hybrid. This technique must be rapid and portable for field use. Another area where research is needed is to develop a non-food, non-toxic attractant that can be used during surveys to determine presence or absence of IFA from the survey area.

The need for a rapid field identification technique that can be used during inspections is also part of IFA Quarantine regulatory research needs. Other regulatory research needs include the development of treatments for a combination of pests such as IFA and Japanese Beetle, root weevils, etc. Research is needed into a fire ant repellant that could be used in the area of regulated commodities to assure that commodities without fire ants don't become infested. In addition, research and development of new pesticides for use against fire ants continues to be needed.

Research into biological control agents is the final area considered in this presentation. From 2002 to 2005 APHIS has enabled phorid fly releases at 51 sites in 13 states. Approximately 43% of those releases have been successful. The two *Pseudacteon* species that have been released to date are P. *tricuspis* and *P. curvatus*. Based on the host preferences of the phorid flies released to date it is clear that many more species of phorid flies will need to be released before a measurable impact can be expected on IFA populations. Therefore, any research about phorid flies is most important, including better survey (e.g. traps, phermones, etc) for field detection of populations. In addition, research into other biological control agents is also viewed as a high priority to APHIS.

Acoustic and High-Speed Videographic Analysis of Flying Imported Fire Ants

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Abstract

We investigated the acoustics associated with the flight of imported fire ants (Black or BIFA: *Solenopsis richteri* Forel, Red or RIFA: *Solenopsis invicta* Buren, and their hybrid). Recordings of tethered alates were generated in an anechoic chamber using a B&K microphone of sensitivity 100 mV/Pa and bandwidth 12.5 kHz, revealing fundamental wingbeat frequencies from 80-130 Hz, with integer harmonics observed as well. The fundamental frequency of RIFA was slightly but significantly higher than BIFA/hybrid regardless of sex, and male alates also had a significantly higher wingbeat frequency than females regardless of species. In addition to the fundamental frequency and harmonics, frequencies were recorded in the high (10,000 to 20,000 Hz) to ultrasound range (20,000 to 50,000 Hz). A large pulse of high frequency was observed for the downstroke portion of the wingbeat cycle, while a smaller amplitude pulse was seen on the upstroke. These sound pulses may be produced by the shedding of vortices from the wingstrokes.

Field recordings of BIFA (or hybrid) male and female alates were performed with ¼ inch B&K microphones. As alates were preparing to fly off grass stems near the mounds, microphones were held ~ 10 mm away to record the take-off flight. These results were similar to laboratory recordings, although much of the data is still being analyzed and more field recordings will be collected in summer 2006.

Eight and sixteen ½ inch B&K microphones of 50 mV/Pa sensitivity and bandwidth 48 kHz were configured as circular arrays (45° and 22.5" separations, respectively) in order to record real-time three-dimensional sound profiles around flying alates. Sound patterns are consistent with those of a dipole source, with some differences observed in the pattern between orders of harmonic, indicating monopole contributions. If the insect can perceive sound at these frequencies, this changing pattern could be used as a cue for the relative orientation of another flying alate. Systematic differences in wingbeat frequencies for males vs. females could also be used as a cue for the sex of the other flying alate.

Stereo high-speed videography (8,000 frames per second) was used to confirm the acoustic wingbeat frequencies and to map the position and shape of the alate wings, with sufficient phase resolution in a wingbeat period to enable an aerodynmanic analysis and numerical simulation of the wingbeat induced flow. This near-field particle flow may be important in alate recognition as it is in some other insects by inducing nerve responses in hair sensilla.

Arkansas' First *Pseudacteon curvatus* Release

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Abstract

Pseudacteon *curvatus*, a phorid fly was released in Clark County, Arkansas in October 2005. This represents the first release of this phorid fly species in Arkansas however Pseudacteon tvicuspis has been released in other areas of the state. Approximately 8,500 Pseudacteon *curvatus* parasitized red imported fire ants were released during this study. The status of this release is unknown at this time but will be evaluated in 2006.

Introduction

Classical biological control attempts to reunite predators or parasites with their prey or host. Arkansas is attempting to reunite the phorid fly Pseudacteon curvatus Borgmeier (Diptera: Phoridae), with its natural host the red imported fire ant, Solenopsis invicta Buren (Hymenoptera: Fornnicidae).

This effort was possible through cooperative efforts involving the University of Arkansas Cooperative Extension Service and USDA's Animal and Plant Health Inspection Service and Agriculture Research Service.

Pseudacteon curvatus is a decapitating fly. Its egg is oviposited by the female into the host and most of the maggot's development occurs in the head of the red imported fire ant. In the process of developing an enzyme is released by the fly which causes the intercuticular membranes of the ant to degenerate and the head falls off.

Mature larvae pupate in the head capsule and remain there until the adult fly emerges (Porter 1998a). However, it is not the parasitic action that makes this fly an effective biological control organism, it is the behavior that red imported fire ants exhibit when flies are present. Red imported fire ant workers are aware of the presence of phorid flies resulting in a cessation of foraging. Inhibition of this foraging occurs as long as these flies are present.

It is hoped that this inhibition will result in increased capacity of native ants to forage for food in imported fire ant territory (Porter 1998a, Porter et al. 2004).

Educational programs in Arkansas have revealed that Arkansans are hopeful that releases of phorid flies will provide long term relief from problems associated with red imported fire ants. Porter et al. (2004) describes releases of biocontrol agents like phorid flies as "the only hope we have for permanent control of imported fire ants in the rural landscape."

The fear that phorid flies will cause environmental havoc shared by a few Arkansans are quickly dampened by descriptions of host specificity studies that have been conducted and reconfirmed by Porter (1998b), Gilbert and Morrison (1997) and Vazquez and Porter (2005).

Materials and Methods

Study Site: The site chosen for the Pseudacteon curvatus release was a pasture / hay meadow located in Clark Co. in southwest Arkansas (Fig. 1). It was bordered by a heavily wooded (hardwood and pine species) area on the west side and deep stream bordered with hardwoods and a moderate density of under story species on the south side.

Data collected from three ¼ acre circles revealed a density of 148 red imported fire ant colonies per acre (Fig. 2). The average fire ant population rating using the USDA rating scale (James 2005) was 15.09 (1000-10,000 workers present) or colony type 3. Workers approximately 3 mm in length were the predominate size in the study area (Fig. 3).

Ant Collection: On 10 October, 2005 ants were collected from 10 colonies and on 18 October, 2005 ants were collected from 7 colonies. One hundred forty eight colonies were flagged and numbered within the study area. Colonies that were used for collections were semi permanently marked with uniquely numbered survey stakes.

Red imported fire ant workers were collected from the selected colonies using the methods described by Roberts and James (2005). Two to three wooden 5/8 inch diameter plant stakes were stuck into the colonies selected for collection (Fig. 4). After a number of workers crawled up the stake it was removed and placed inside a plastic bucket with a fluon coating on the upper interior of the bucket to prevent ant escape. Workers were knocked off the stake and into the bucket by gently tapping the stake with another stake (Fig. 5). This process was repeated numerous times for each mound until approximately 3-4 grams of ants were collected (Fig. 6). After 3-4 grams of ants were collected they were transferred to sandwich boxes containing a fluon barrier and nesting tubes (Fig. 7). This was achieved by tilting the bucket and hitting its' sides to consolidate ants into a single clinging mass. This mass of ants was then quickly transferred (dropped) into the sandwich box. Each sandwich box was labeled with the identical number as the colony the ants were collected from.

Sandwich bags containing the ants were sealed with tape, placed in cotton pillow cases and placed into a large insulated shipping cooler with paper wrapped ice packs. The cooler containing the ants collected that day were shipped overnight to Debbie Roberts in Gainesville Florida to be parasitized by Pseudacteon curvatus.

Ant Release: Red imported fire ants received by the lab in Gainesville, Florida were first allowed to acclimate and then subjected to parasitism by Pseudacteon curvatus for approximately 48 h. Parasitized ants were shipped back to Arkadelphia, Arkansas by overnight carrier. As soon as ants were received in Arkadelphia they were transported to the release site. Parasitized ants were returned to the same colony from which they were collected (Fig. 8). Ants collected on October 10 were returned to the colony on October 18. Likewise, ants collected on October 18 were returned to the colony on October 25.

Results and Discussion

During the summer and fall of 2005 the most severe drought since 1958 occurred in southwest Arkansas. Also during mid October temperatures were cooler than normal and included several mornings with frost.

Despite watering fire ant colonies the day prior to collection, the desired quantity of red imported fire ants was not collected from every colonies. However, based on the quantity of ants collected, we estimate that approximately 8,500 parasitized ants were returned to the study site during in October 2005. We will begin evaluating the release in the spring and summer of 2006.

If it appears that Pseudacteon curvatus did not establish at this site it could be related to the drought conditions and future releases at this site may prove successful.

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Thanks to Debbie Roberts, USDA APHIS, PPQ, CPHST, in Gainesville, Florida for supplies used in the release and parasitization of the ants; Sanford Porter, USDA-ARS Gainesville, FL for our initial phorid release training; Anne-Maria Callcott, USDA-APHIS, Gulfport, MS and Joel Bard, USDA-APHIS, Little Rock, Arkansas for cooperative efforts and support of this project.

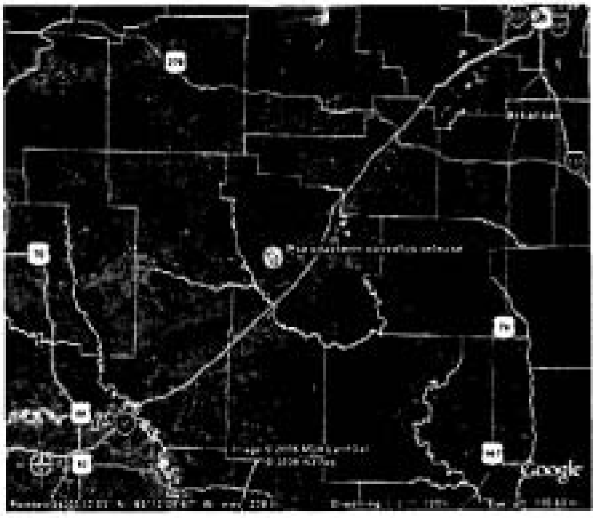

Fig. 1. Location of study site.

Fig. 2. Flagged colonies at study. Note density of colonies.

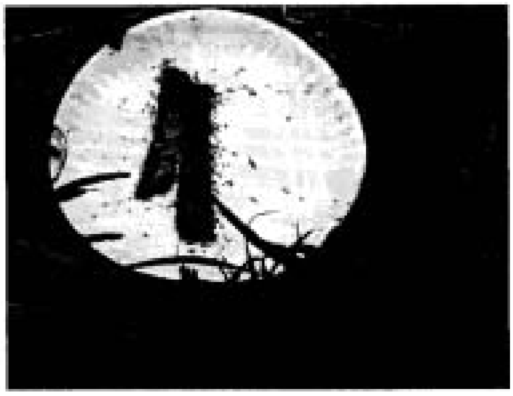

Fig. 3. Red imported fire ants from study site. Note predominant size

Fig. 4. Red imported fire ants crawling up plant stake

Fig. 5. Dislodging ants from plant stakes.

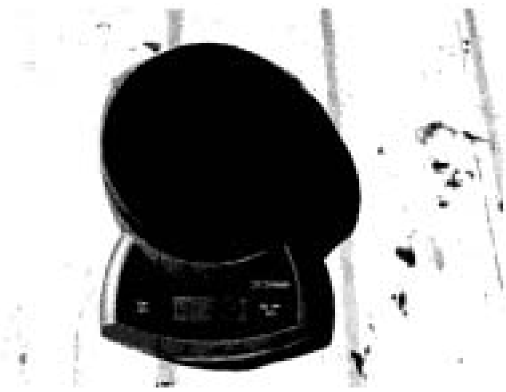

Fig. 6. Digital scales used to weigh ants

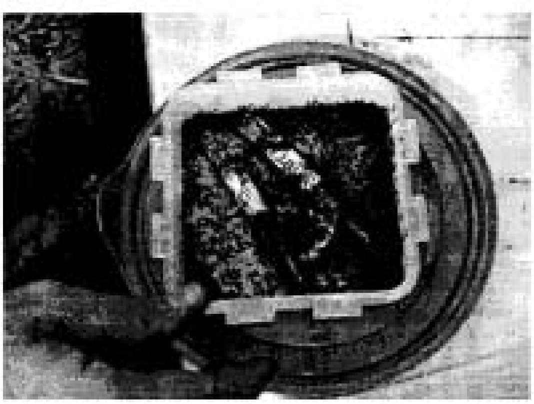

Fig. 7. Sandwich box containing ants for shipment to Gainesville, Fla.

Fig. 8. Releasing parasitized red imported fire ants into colony.

Distribution Patterns of *Thelohania solenopsae* in the RIFA Populations of Southeastern Oklahoma: Implications

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Abstract

Thelohania solenopsae, a microsporidian parasite of the Red Imported Fire Ant (RIFA), *Solenopsis invicta,* is studied as a potential biocontrol agent. The patterns of occurrence of *T. solenopsae* in the populations of RIFA in Oklahoma are reported in this study. RIFA samples were collected and analyzed twice a year, since fall 2002, from two sites at Bryan County and one site at McCurtain County. Although earlier attempts to release and establish this pathogen on RIFA population in Oklahoma had failed, *T. solenopsae* have been detected in the workers of *S. invicta* collected at Bryan County since 2002. These infections were confirmed by gene amplification (PCR) and modified Trichrome staining. The RIFA samples tested positive for *T. solenopsae* at varied proportions in the two sites at Bryan County ranging from 37% to 54% in spring (2003 and 2005) and ranging from 27% to 89% in fall (2002 to 2005). The RIFA population in McCurtain County was devoid of the microsporidian parasite except for a short period in fall 2004. RIFA workers from three mounds at McCoy Ranch tested positive for the protozoan parasite when sampled in September 2004, but did not prevail in 2005. These annual fluctuations in the occurrence of the parasite in the fire ant population may indicate that *T. solenopsae* is in the process of establishing in fire ant population in Oklahoma.

Introduction

The Red Imported Fire Ant (RIFA), *Solenopsis invicta* was accidentally introduced into the United States in the 1930s. Since then, this species has spread and established in almost all southern states and is gradually moving northwards. **A** major factor attributed to its successful survival is the absence of natural enemies in the non-native environment. RIFA infests over 112 million hectares in the United States (Lofgren, 1986) and has impacted the native insect species and other arthropods enormously through competition for resources. *S. invicta* is more abundant in the United States than in its native habitats in South America (Porter *et al.,* 1992), chiefly due to the lack of natural enemies in the introduced habitats. In Oklahoma, *S. invicta* is chiefly confined to the southern counties, though several northern counties have been infested with RIFA from the summer of 2004 onwards (unpublished data). Recently, researchers have been focusing on efficiency of sustainable control methods, which mainly include biological control agents. T. *solenopsae,* a microsporidian obligate intracellular pathogen has been an effective natural enemy against the fire ants in Argentina (Briano *et al.,* 1995). *T. solenopsae* declines the egg production, queen weight and survivability of queens and workers of *S. invicta* (Oi and Williams, 2002). In this study, we have monitored the proportion of RIFA population that was infected with T. *solenopsae* at three improved pastures, *viz.,* Adam Ranch and Bowles Ranch at

Bryan Co. and McCoy Ranch at McCurtain Co. The patterns of occurrence of this infection from 2002 to 2005, in spring and fall, have been compared.

Materials and Methods

Fire Ant samples were collected from three sites – two from Bryan Co., OK and the other from McCurtain Co., OK (Fig 1). These sites are part of our Areawide Fire ant management project. Each site has several plots and samples were collected from 3-5 mounds in each plot that had fire ant mounds. The samples were tested for the presence of T. *solenopsae* spores using PCR and modified Trichrome staining.

Polymerase Chain Reaction:

Fifteen to thirty ants were ground with 500µl TBS using a disposable pestle. To this homogenate, 0.1mm glass beads were added up to three quarters of the tube and beaten at maximum speed for 15 seconds, in a bead beater. The tubes were immediately transferred to a 95°C water bath for 5 min. The samples were spun for 10sec at 18000g and the supernatant containing genomic DNA of *T. solenopsae* was collected.

2µl of the total DNA was added to the mixture of *T. solenopsae* specific primers - Mspla and Msp4b (lpl each) and Ready-to-go-beads. This mixture was made up to 25µl with sterile distilled water and the PCR reactions were set as described by Snowden *et al.*, 2002. PCR products were separated on 2% agarose gels and visualized after staining with Ethidium bromide (Fig 2). For all experiments, positive (T. *solenopsae* DNA) and negative (PCR reaction mixture without total DNA) controls were also run along with treatments.

Trichrome staining:

Fifteen to thirty ants were ground with 500µl TBS using a disposable pestle. About 15µl of the ground solution is placed on labeled glass slide and air-dried. The slides were stained using the modified Trichrome staining protocol of Kokoskin *et al.* (1994). The slides were observed under a light microscope at 400x and 1000x magnifications for T. *solenopsae* spores (Fig 3).

Results and Discussion

The two sites at Bryan Co. had RIFA population with *T. solenopsae* infections, since fall 2002. The source of this protozoan pathogen found in 2002 RIFA samples in Oklahoma is unknown, as two earlier attempts (1998 and 2000) to establish this pathogen in Bryan and Carter Counties, in S. *invicta* mounds failed. Interestingly, higher percent of RIFA population were infected with T. *solenopsae* every fall since 2002 and a relatively lesser population was infected in spring with the exception of 2005 (Fig 4). The least proportion of infected populations was observed during fall 2005 at Adam Ranch (27.3%) and the highest proportion of 75% in the fall of 2002 and 2004. At least 36.8% of the fire ant population sampled was infected in spring 2003 at Bowles Ranch, with maximum being 89.5% infected RIFA workers in fall 2002. In spring 2003, the infected RIFA workers were fewer in Adam and Bowles Ranches (43.8% and 36.8%, respectively). However, by spring 2004, the infected population increased to 53.9% at Adam Ranch and 50% at Bowles Ranch. All the RIFA samples collected from the site in McCurtain Co. were free of T. *solenopsae* infections except during fall 2004. Three mounds were infected with the microsporidian pathogen, but never prevailed until spring 2005. In Oklahoma, monitoring of the pattern of spread of this pathogen in the RIFA population, along with the monitoring of the pattern of spread and establishment of *S. invicta* itself is imminent. A long-term monitoring study has been taken up to help us understand the strategies adopted by an invasive species (here, *S. invicta)* to survive and establish in an introduced region in spite of the ecological pressures.

The low proportion of infected RIFA population observed almost every spring may indicate that the infected colonies perish during the winter and that the mating flights occurring between spring and summer has to repopulate the sites with infected and uninfected colonies. The annual turnover may depend on the severity of the winter and the ensuing summer conditions.

Acknowledgments
We thank Wayne Smith and Richard Earnhart for the help with the collection of *S. invicta* samples at the three study sites. VK thanks Forrest Mitchell for the training with PCR and modified trichrome staining methods.

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Fig 1. Study Sites

Fig 2. PCR products separated in 2% agarose gel. M is the molecular weight marker lane and lanes 1 and 2 are positive and negative controls, respectively. The bands seen in the lanes 3, 6 and 8 are RIFA workers with *T.* solenopsae spores in Bowles Ranch. Samples from other lanes tested negative for this pathogen.

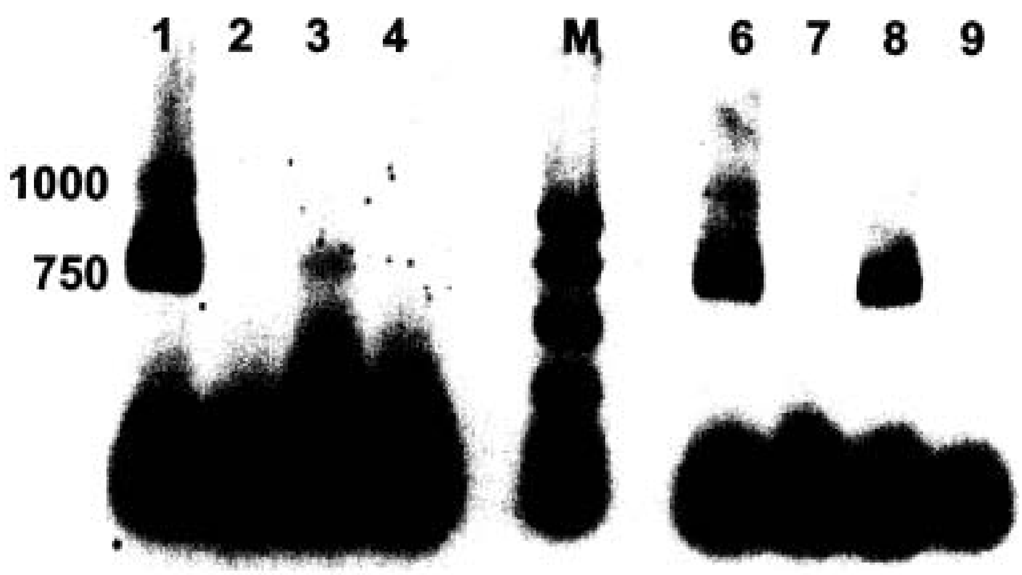


Fig 3. Photomicrograph of binucleate free spores detected from RIFA samples

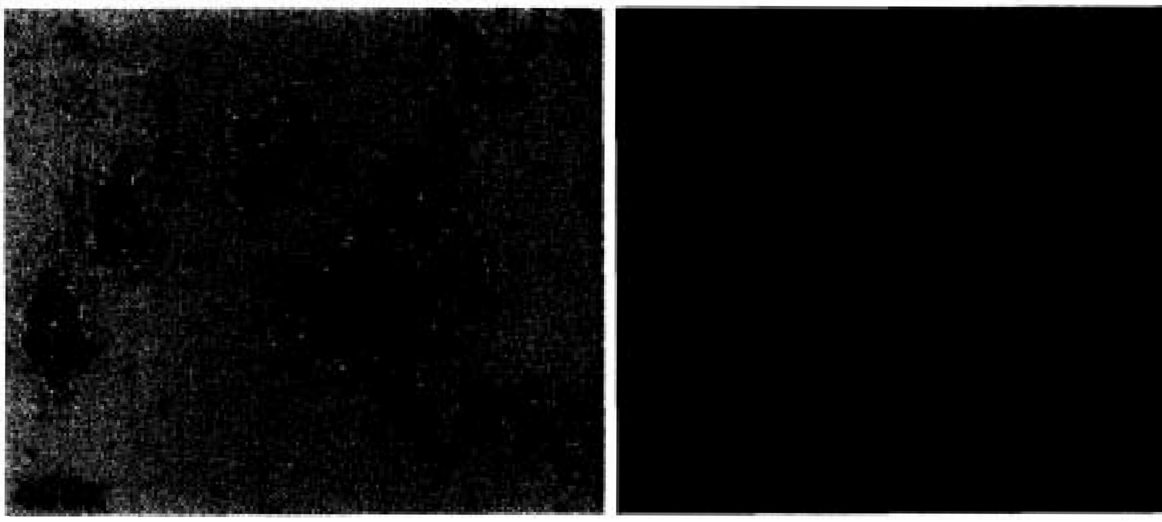
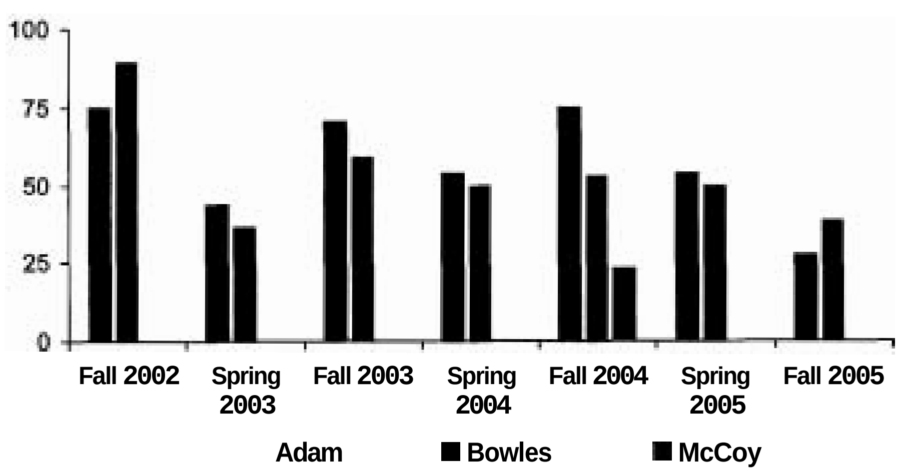


Fig 4. Variability in the percent of RIFA workers infected with *T. solenopsae* in southeastern Oklahoma



Host Location Behavior of *Pseudacteon curvatus* (Diptera: Phoridae) in Mono- and Polygyne Fire Ant Colonies (Hymenoptera: Formicidae)

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Introduction

Imported fire ants, Solenopsis richteri and Solenopsis invicta, are accidentally introduced invasive species that are native to South America. One reason fire ants have been so successful and have spread so rapidly is their natural enemies were left behind in South America (Jouvenaz 1990). In an attempt to tip the ecological balance in favor of native ants and to reduce imported fire ant abundance, a parasitoid has been introduced into fire ant populations in the United States (Williams et al. 2002; Porter 1998b). The parasitoid is a dipteran that attacks Solenopsis fire ants. In 14 Alabama counties, three species of phorid fly have been released: Pseudacteon tricuspis, Pseudacteon *curvatus*, and Pseudacteon litoralis. Morrison and King (2004) examined the host location behavior of P. tricuspis in Florida. This study is designed to study the behavior of P. *curvatus* in Alabama.

Materials and Methods

A series of field experiments were conducted using P. *curvatus* modeled after the methods Morrison and King (2004). The study was located at two sites (a monogyne site and a polygyne site) in Talladega County, Alabama.

"bait" consisted of a 4 g section of hot dog placed on a 5 x 5 cm laminated card (fig. 1). Baits were spaced 5 m apart on one or more transects. When a "disturbance" was applied, a crater about 20 x 20 cm was dug into the mound using a small shovel.

When a "non-nestmate" (NNM) disturbance was applied, a cup with a predetermined amount of ants from a laboratory colony were applied to ants either at a bait and/or in a mound.

When a “shock” treatment was applied, it was administered to ants in a mound using a modified electric cattle prod similar to one developed by Charles Barr (fig. 2).

Figure 1: Hot **Dog Bait**

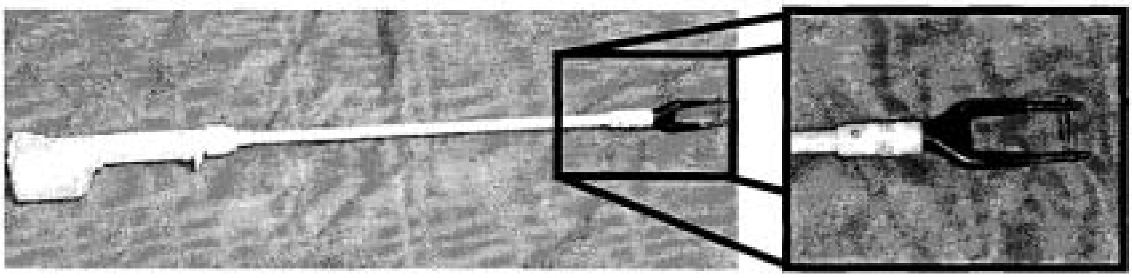


Figure 2. Modified cattle prod used to apply electrical "shock" treatment.

Experiment 1: Are P. *cuwatus* more attracted to workers at a disturbance or to workers at a food source? For the first part of the experiment, 40 baits were placed 5 m apart on two transects. After one hour of recruitment time, fly presence was monitored at 20 minute intervals for one hour. For the second part, 30 mounds were disturbed by digging a crater as previously described. After 10 minutes, fly presence was monitored at 10 minute intervals for 30 minutes.

Experiment 2: Are flies more attracted to workers that are simply foraging or to workers that are competing at a food source? Forty baits were placed 5 m apart on two transects. After one hour of recruitment time, every other bait on the transect received a 200 NNM treatment. Fly presence was monitored at five minute intervals for 20 minutes.

Experiment 3: Does increasing the interaction at a food resource affect the number of flies attracted? A total of 30 baits were placed on 5 transects 5 m apart. After one hour of recruitment time, treatments of five, 25, 50, 100, and 250 NNM were randomly assigned to five replicates in a Latin Square Design. Fly presence was monitored at five minute intervals for 30 minutes, then at 10 minutes intervals for an additional 30 minutes for a total time of one hour.

Experiment 4: Are flies more attracted to workers at colony disturbances with interaction or to workers at colony disturbances without interaction and then, are they differently attracted to the interactions? Fifteen mounds were disturbed. To the first five mounds, no additional treatment was added. To the next five mounds, a 15 second shock was administered via the modified cattle prod. To the last five mounds, a 300 NNM treatment was administered. All 15 mounds were monitored at 10 minute intervals for 30 minutes.

Results

Experiment 1: Are P. *curvatus* more attracted to workers at a disturbance or to workers at a food source?

Monogyne site - The baits had very low fly numbers at each collection time (zero flies 115 out of 116 observations). However, at the disturbed mounds, flies were found at a higher density at all times (flies present 46 times out of 90 observations). Significantly more flies were found at disturbances over the 30 minute monitoring period as opposed to the foragers at baits over 60 minutes ($p<.0001$). The foraging ants attracted flies to one of 39 baits over the one hour monitoring period while the ants at the disturbed mounds attracted flies to more than 14 of 30 mounds over the 30 minute monitoring period.

Polygyne site - The baits had very low fly numbers at each collection time (zero flies 119 out of 120 observations). As at the monogyne site, flies were found at a higher density at all times (flies present 60 times out of 90 observations) at the disturbed mounds. The difference in total numbers of flies present at the disturbances over the 30 minute monitoring period as opposed to foragers at baits over the 60 minute was highly significant ($p<.0001$). The foraging ants attracted flies to one of 40 baits over the one hour monitoring period while the ants at the disturbed mounds attracted flies to 10 of 30 mounds over the 30 minute monitoring period.

Experiment 2: **Are flies more attracted to workers that are simply foraging or to workers that are competing at a food source?**

Monogyne site – At five, ten, and 20 minutes, fly numbers were too low to use Chi-Square as an accurate test. When fly numbers are totaled for all collection periods, significantly ($p=.0020$) more flies were found at bait with NNM interactions than at baits with only foragers (fig. 3).

Polygyne site - At five, ten, 15, and 20 minutes, there is a significant difference between baits that have a conspecific interaction and baits that only have foragers ($p=.0312$, .0384, .0012, and .0001). More flies were found at baits with NNM interactions than at baits with only foragers at 5, 10, 15, and 20 minutes and for total number of flies (fig. 3).

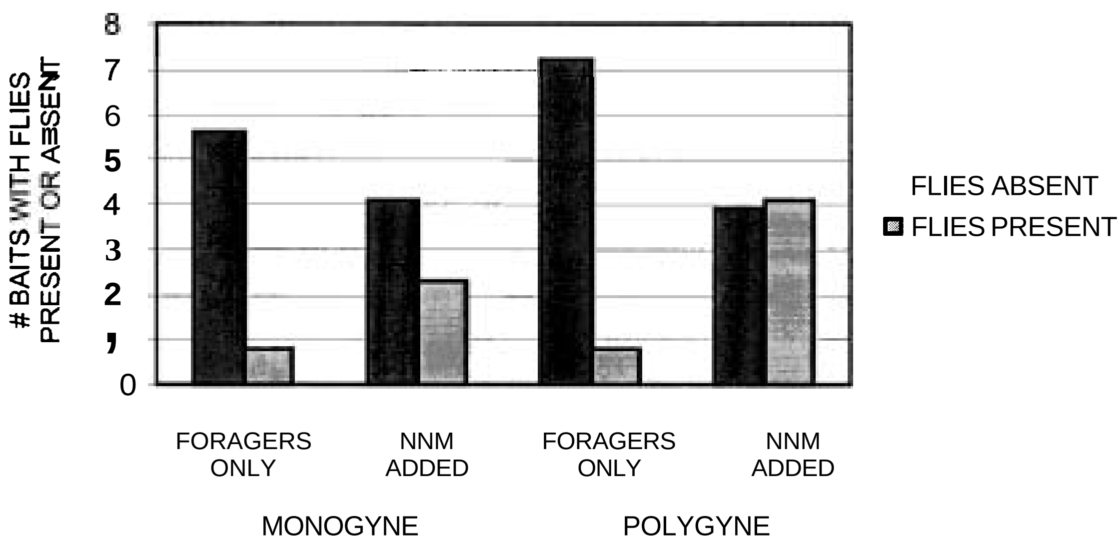


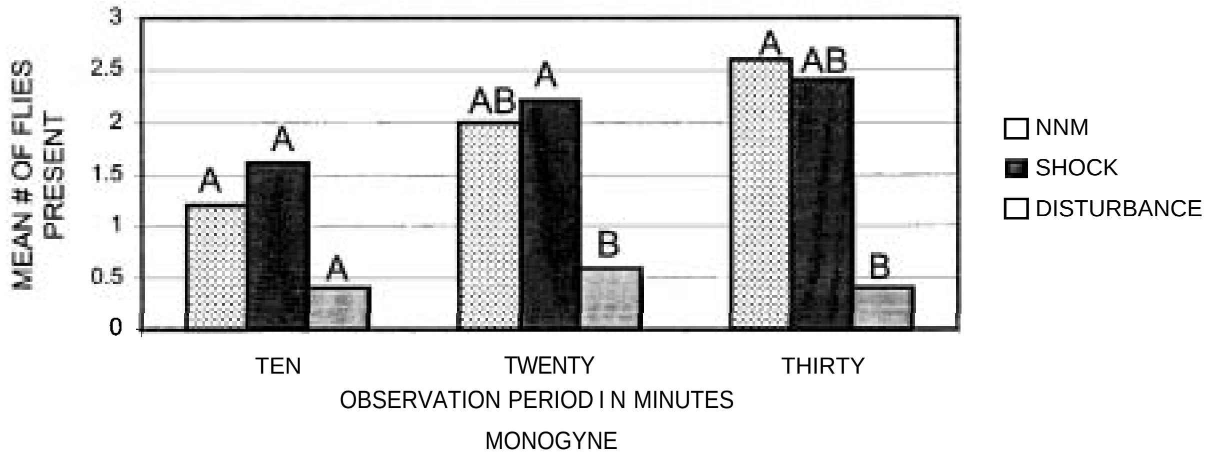
Figure 3 Total fly activity at baits with NNM added or foragers only.

Experiment 3: Does increasing the interaction at a food source affect the number of flies attracted?

Monogyne site – There was no significant difference in fly numbers between the 25 and 100 NNM treatments. However, significantly more flies were found at the 5 and 50 NNM treatments than at the 250 NNM treatment.

Polygyne site – There was no significant difference between fly numbers at the 5, 25, 50, or 250 NNM treatments. However, significantly more flies were found at the 100 NNM treatment.

Experiment 4: Are flies more attracted to workers at colony disturbances with interaction or to workers at colony disturbances without interaction and then, are they differently attracted to the interactions? There were no significant differences between the three treatments at ten minutes at the monogyne site. However, at the polygyne site, there were significantly more flies at the NNM and the shock treatments than at the disturbance. At both sites, there were significantly more flies at the NNM treatment at 30 minutes than at the disturbance. However, at 30 minutes at both sites, there was no difference between the shock treatment and the NNM or disturbance treatments. At the monogyne site at 20 minutes, significantly more flies were found at the shock treatment than at the disturbance (fig. 4).



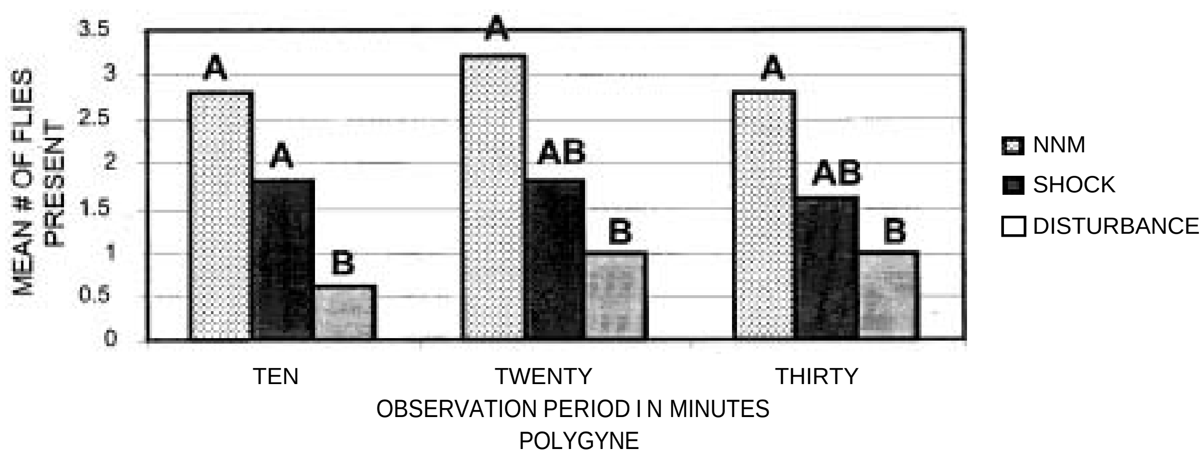


Figure 4 Mean number of flies attracted to NNM treatment, shock treatment, and disturbance only.

Discussion

Pseudacteon cuwatus in Alabama are more attracted to fire ants at a colony disturbance (whether it is a mound disturbance or a NNM disturbance) rather than to fire ants at a food source. While phorids are attracted to NNM disturbances, attraction was not enhanced by increasing the number of NNM. *Pseudacteon cuwatus* are differently attracted to colony disturbances with and without interactions. In both monogyne and polygyne colonies, they are least attracted to a mound disturbance alone. In polygyne colonies, attraction is greatest at NNM interactions

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Pseudacteon spp. (Diptera: Phoridae) Range Expansion in Alabama

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Introduction

When fire ants were introduced into Alabama in the early 1900's, almost all of their natural enemies were left behind in South America (Jouvenaz 1990). As a result, fire ant densities are much higher in Alabama than they are in South America (Porter et al. 1997). Two species of imported fire ant occur in Alabama. The red imported fire ant, *Solenopsis invicta,* is located in the southern portion of the state and the black imported fire ant, *Solenopsis richteri*, is located in northwest Alabama.

Although the black imported fire ant was introduced into the United States before the red imported fire ant, its current range is thought to be northeastern Mississippi and northwestern Alabama. Vander Meer et. al. (1985) first detected a hybrid between the two species in Mississippi. The hybrid is thought to populate the northern tier of Alabama, Mississippi and Georgia .

One group of natural enemies that have shown some promise in the battle against fire ants are phorid flies (Figs. 1) in the genus *Pseudacteon* (Porter 2000). Currently, eleven populations of phorids have been successfully established in Alabama (Fig. 2). *Pseudacteon tvicuspis* shows a strong preference for *S. invicta* and is established at seven sites in South Alabama. *Pseudacteon cuwatus* shows a strong preference for *S. vichtevi* and the hybrid fire ant. It is established at four sites in North Alabama (releases in Madison and Lauderdale Counties by K. Ward and R. Ward). *Pseudacteon litoralis* was released in Wilcox County in 2005, but has not been recovered in the field.

Materials and Methods

Releases of *P. tvicuspis* and *P. cuwatus* were conducted as described by Graham et al. 2003. The first successful release was in 1999 and new releases have been conducted yearly in different counties (Fig 2).

A release site and a corresponding control site approximately 9.5 km apart were selected in Macon and Talladega. In Houston, Lowndes, Walker and Cullman, the release and control sites were ca. 32 km apart. Sampling areas for population data were set up in conjunction with each site. Data collected in each sampling area were total number of mounds and mound size.

Pseudacteon litoralis was release in Wilcox Co. using the methods described for *P. cuwatus*.

Results

Mound data are presented from the three oldest release sites in Fig. 3. Droughts occurred in Alabama in 1999,2000, and 2001. In addition, the coldest November and December on record were recorded in 2000. These environmental factors have influenced fire ant populations, as evidenced by the low number of mounds in May 2001 in Macon Co. and reduction in mound numbers in 2001 and 2002 at the Talladega Co. control site, where no phorids had been found at that time. Mound data in Fig. 4 are from release sites with no corresponding control.

Except for the Lowndes Co. release site (Fig. 3), although mound numbers change during the year most sites appear to have fewer fire ant mounds than were present when flies were released or found. Low mound numbers the last **two** sampling periods at the Lowndes Co. release site are a result of the growth of pine seedlings planted at the site. The site will be discontinued this year.

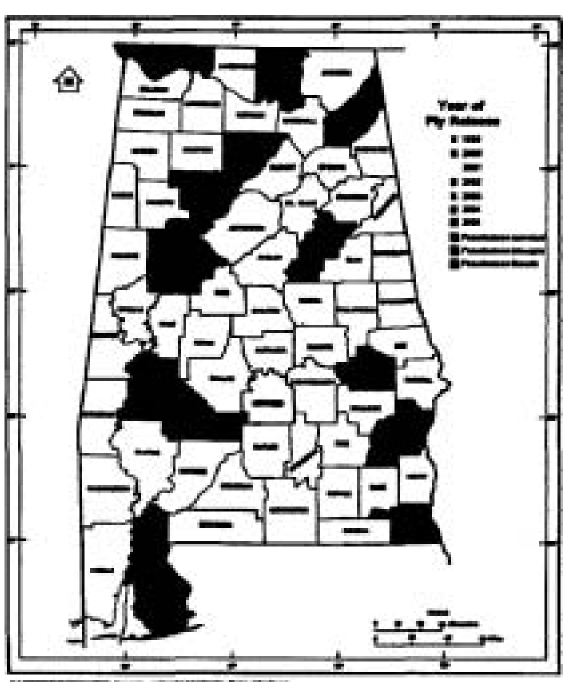
P. tricuspis reached the Macon Co. control site in mid- to late-summer of 2000. *P. cuwatus* were found approximately 1.6 kilometers north of the control site in Talladega in August 2002, but were not found south of the site until 2004. Flies from the Houston and Cullman Co. sites have not been found at the control sites. Flies have not been found at the Lowndes Co. control site, but have been found several miles past it.

The three *Pseudacteon* spp. released in Alabama have been recovered at eleven of fourteen release sites, although they have not been found recently near the Houston and Baldwin Co. sites. Phorids are spreading rapidly across the state and into Georgia (Fig. 5). The ovals estimate the currently mapped ranges of the active populations (Fig. 5). All ranges are underestimates. We have not found the leading edges of any of these populations.

P. *tricuspis* (Fig 5, blue circles) and P. *curvatus* (Fig 5, yellow circles) have expanded their ranges to cover approximately two-thirds of Alabama and the spread in Georgia and possibly Tennessee from is unknown. In addition, flies from the release in eastern Tennessee may now be in northeast Alabama (Fig. 5, red circle). Both phorid species now reproduce in areas occupied by the hybrid and red imported fire ant.

Even though it appears that phorid flies may have affected the fire ant populations at these sites, further study will be required to determine if these population reductions are permanent and due to the flies and not other factors.

Sites in the area where *P. curvatus* and *P. tricuspis* now coexist (Fig 5, green dotted circle) have been monitored since 2003, when no flies were present. Hopefully, the presence of more than one species of these parasitoids in an area will reduce fire ant numbers more dramatically and permanently than the reductions seen in Figs. 3 & 4 above and we will be able to document a long-term reduction in fire ant mounds.



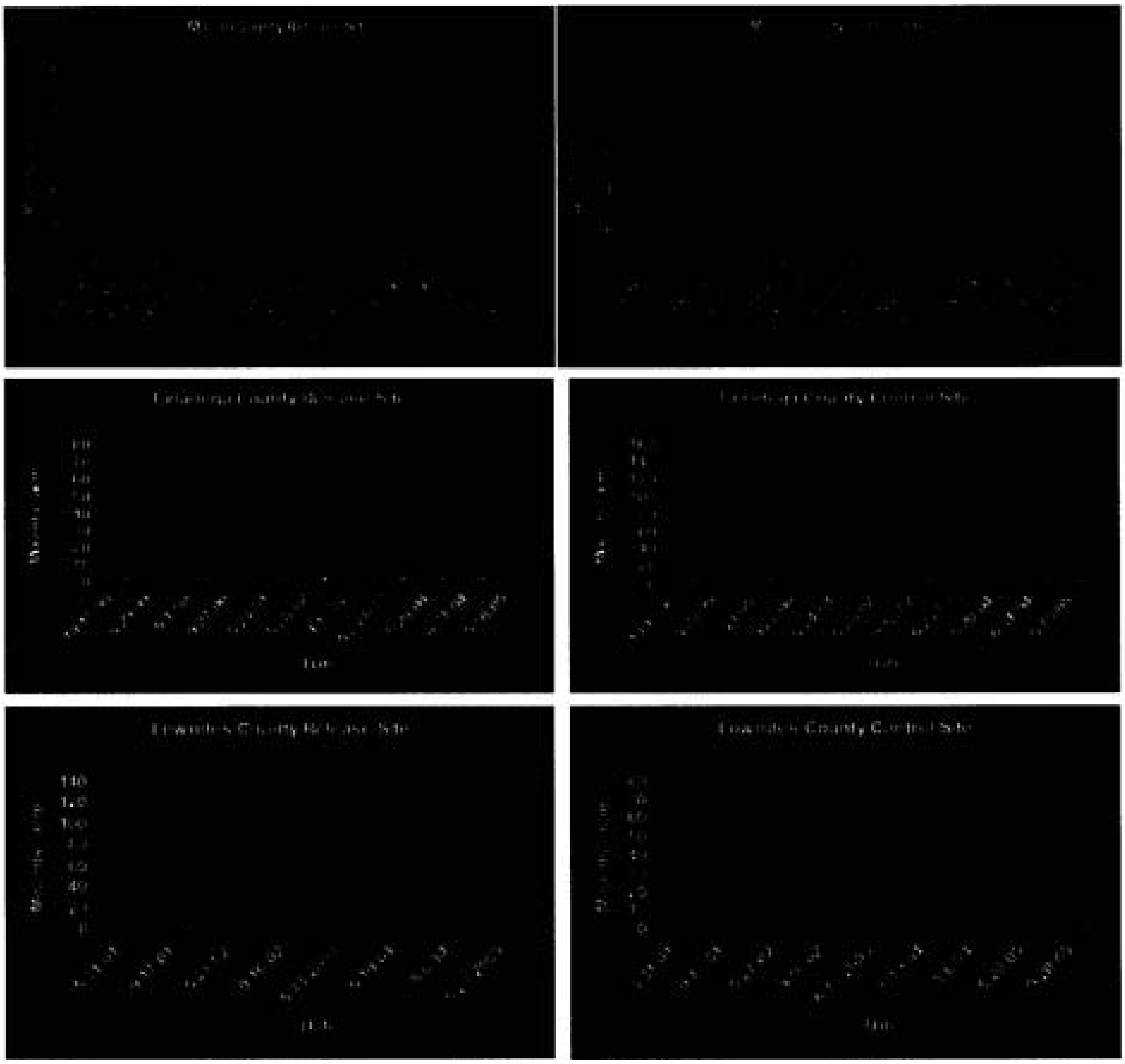


Fig. 1. Fire ant population data from the first three phorid releases and corresponding release sites. White arrows indicate when flies were found at release sites.

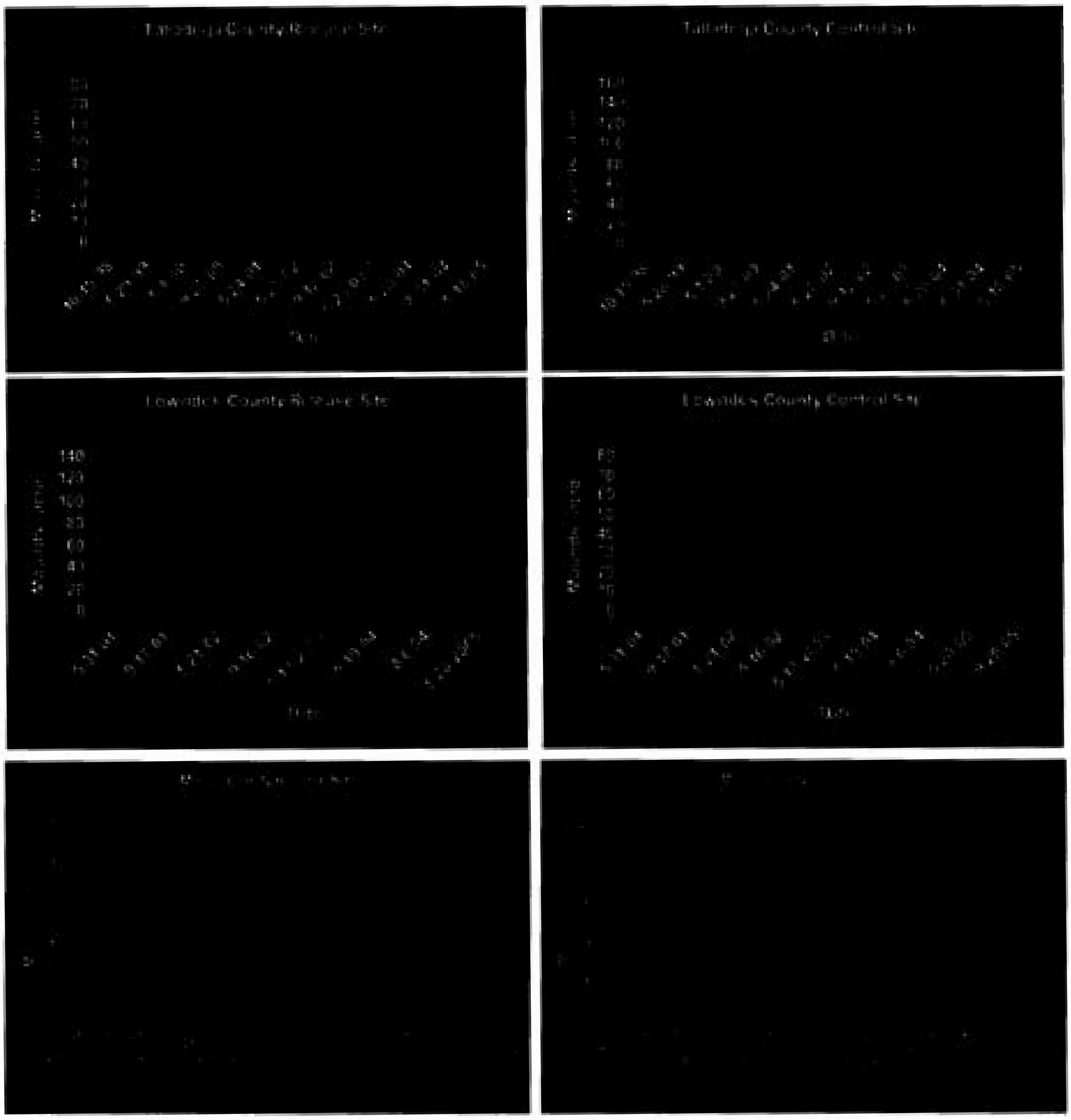


Fig. 2. Fire ant population data from release sites only. First data point on each graph corresponds with phorid fly release date.

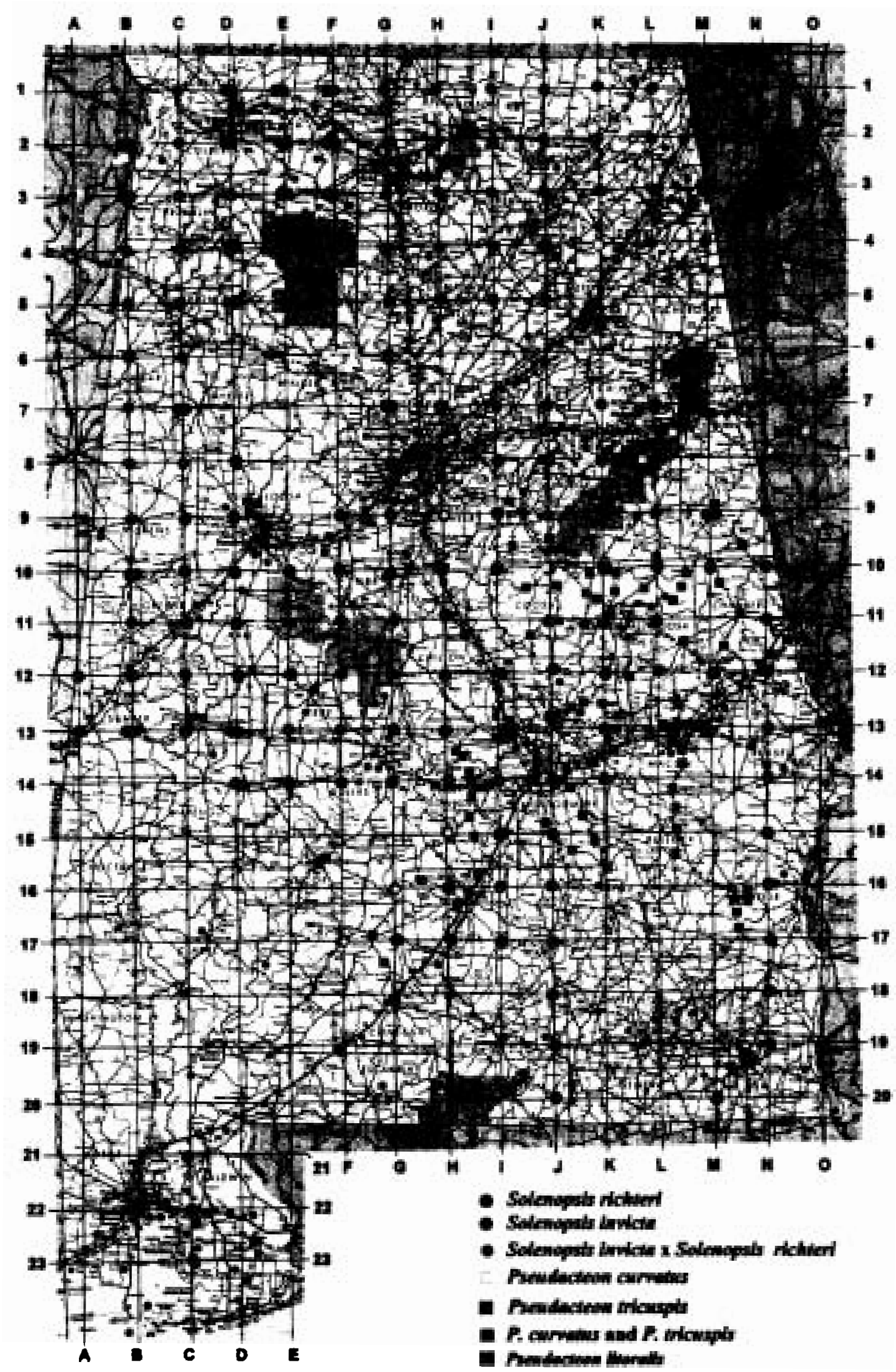


Fig. 3. Range of *Pseudacteon* and *Solenopsis* spp. in Alabama

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Current USDA-APHIS Phorid Fly (Pseudacteon spp.) Efforts in Imported Fire Ant Populations

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Introduction

A recent USDA-APHIS survey indicated that seven southern states ranked imported fire ants, Solenopsis invicta, Solenopsis richteri and Solenopsis invicta x Solenopsis richteri hybrids, (IFA) as a top priority target organism for biological control. In response, APHIS has begun collaborative efforts with other federal, state, and university organizations to release decapitating phorid flies (Pseudacteon spp.) from South America against IFA. There may be as many as 15 species or biotypes of the fly that are relatively specific to IFA. The goal of this project is to have reproducing flies of several approved species and/or biotypes of flies at approximately 2-5 sites per IFA-quarantined state including Puerto Rico. Once set in motion, these self-sustaining biological interactions will hopefully negatively impact IFA and allow the agricultural community and others, to reduce chemical control dependence.

Methods

USDA-ARS-CMAVE, Gainesville, FL is responsible for natural enemy exploration in South America, country clearances, and preliminary releases. Also, CMAVE is responsible for initial mass-rearing methodologies. Adequate rearing techniques and production duties are transferred to Florida Dept. of Agriculture, Division of Plant Industries (DPI), Bureau of Methods Development and Biological Control in Gainesville, FL. Flies are shipped directly from the rearing facility as either IFA head capsules containing pupae or infected live ant colonies to cooperators for release. Cooperators handle releases and monitoring. USDA-APHIS-CPHST, in Gulfport, MS is responsible for all coordination between cooperators and the rearing phase.

In a related APHIS project, phorid fly releases, establishment, and spread are being monitored through the development of a Geographic Information Systems (GIS) decision support and management program for phorid fly monitoring and evaluation. For this aspect of the project, APHIS requests cooperators to collect IFA and fly data. Cooperators conduct pre-release IFA population evaluations and repeated biannual counts. Cooperators establish and collect fly information at each release site and document spread through bi-annual remote site evaluations. Several data collection options have been developed; 1) paper forms, 2) hand-held PDA units and software, and 3) and online data entry web-site.

Detailed information about the several aspects of the project can be viewed at the following web-sites: CPHST-ANPCL-SIPS phorid rearing webpage: http://www.cphst.ovg/projects/Phorid–rearing/, FL-DPI phorid fly rearing webpage: http://www. *doacs.state.fl.us/pi/methods/fire-phorid.*htm, and CPHST-ANPCL-SIPS online data entry site: https:/flydata. cphst.*org/flyData/main.*cfm.

Results and Discussion

Currently two species of flies are being reared and released in the APHIS program Pseudacteon tricuspus and P. cuwatus. (Table 1). Pseudacteon tricuspis prefers red IFA (ca. 9 - 7 boxes in rearing) while Pseudacteon curvatus prefers smaller red IFA (polygyne) ca. 5 - 7 boxes in rearing. Two additional species (P. littoralis and P. obtusus) are being considered rearing for 2006-2007. We are shifting production to more P. *curvatus* over P. tricuspus. P. tricuspus has been released widely and P. *curvatus* appears to better on black and polyygne IFA. Between 2002 and 2005, 12 IFA infested U.S. states and Puerto Rico have released at least one species flies (Figure 1.).

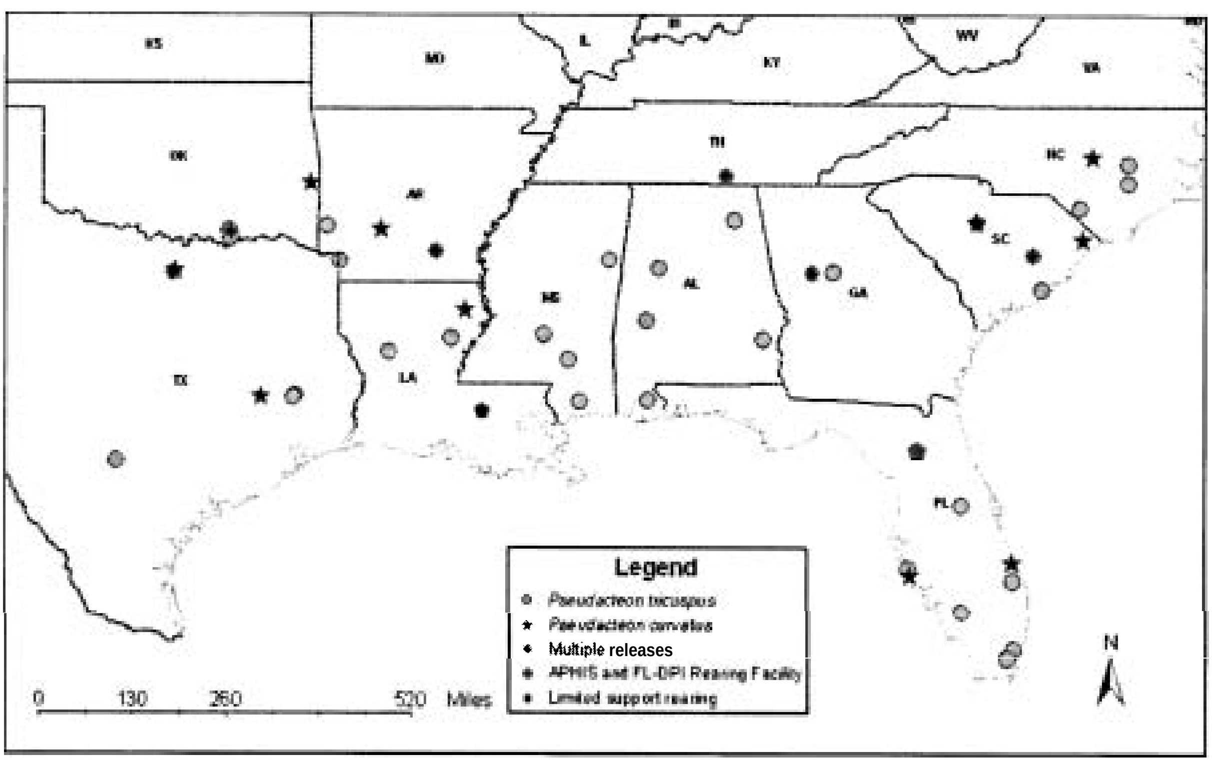
Table 1. USDA APHIS phorid fly production and release numbers between 2002 – 2005.

Fly species Year	Flies produced	Potential flies shipped[a]	Field releases	Mean flies per release
Pseudacteon tricuspis				
2002	942,659	58,750	12	4,895
2003	1,625,067	81,450	15	5,430
2004	1,698,942	89,050	9	9,894
2005	1,381,650	91,175	10	9,117
Pseudacteon cuwatus				
2002	7,404	0	0	0
2003	121,316	0	0	0
2004	581,097	39,552	3	13,184
2005	1,383,641	88,638	7	12,662

[a] Estimated number of pupae in head capsules for P. ***tricuspus*** or estimated number of flies in parasitized live ants in ***P. cuwatus.***

*Totals do not include >88,000 flies distributed for research, education, and other limited rearing facility support (2002 – 2005).

Figure 1. USDA, APHIS, PPQ, CPHST Phorid fly rearing and releases 2002 – 2005. California (2005) and Puerto Rico (2002,2005) releases not shown on this map.



Cloning, Sequencing, and Expression of Arginine Kinase Gene in the Red Imported Fire Ant, *Solenopsis invicta* Buren

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Abstract

The availability of biochemical energy, with ATP as the primary energy currency, is fundamental to most cellular processes. Although ATP and its congeners are involved in literally hundreds of biochemical reactions, the intracellular concentration of ATP is generally kept very constant. Hence, metabolic ATP generation in a cell must be balanced tightly with ATP-consuming processes. This balance between energy-consuming and -producing processes is particularly challenged in tissues that experience periods of high and fluctuating energy demand, such as brain or muscle. To maintain constant ATP levels, these tissues express phosphagen kinases that synthesize a metabolically inert pool of phosphorylated compounds (phosphagens) during normal metabolic conditions and to replenish the ATP from this pool during periods of high energetic demand. Eight phosphagen kinases are found in the animal kingdom, with arginine kinase as a prominent example, occurring in insects. Arginine kinase plays an important role in cellular energy metabolism in invertebrates. Recently, an arginine kinase gene was cloned and sequenced from the red imported fire ant. The cDNA sequence of the gene has open reading frames of 1065 nucleotides encoding a protein of 355 amino acid residues. Northern blot analysis was performed to compare expression levels of the arginine kinase gene for different developmental stages, castes, and specific tissues of the fire ant. We demonstrate that the expression of the arginine kinase gene is developmentally, caste specifically, and tissue specifically regulated in red imported fire ants. Levels of arginine kinase mRNA were readily detectable in $3^{rd}+4^{th}$ instars, worker pupae, and alate (mixed sex) pupae; increased in male and the female alates; and rose to a maximum in workers. In the female alates, the expression of arginine kinase mRNA was very low in abdomen, increased in thorax, and reached a maximum in head. Whereas, in worker, the expression of arginine kinase mRNA was very high in both thorax and head. These results suggest that the tissues that experience periods of high and fluctuating energy demand are different between worker and female alate.

Diel and Seasonal Phenology, and Spatial Dynamics of *Pseudacteon tricuspis* Borgmeier (Diptera: Phoridae) in Louisiana

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Monthly population surveys of *Pseudacteon tricuspis* were conducted at two locations in SE Louisiana: –5 km NE of Covington (St. Tammany Parish) and –10 km E of Norwood (East Feliciana Parish). Surveys were carried out within three plots (plots are 0.5 ha, and >100m apart), and all three plots were sampled on an hourly basis. All flies that appeared at five randomly selected *Solenopsis invicta* mounds within a two-minute observation period per mound were counted. Surveys were normally done between 10 AM and 5 PM (CDT). In general, peak *P. tricuspis* activity occurs between 1 and 2 PM (CDT), at approximately the time of solar maximum. Occasionally a second, late afternoon, peak would be observed. The seasonal dynamics of *P. tricuspis* at our two survey locations in Louisiana were ascertained using the data collected from the surveys described above and data consisting of the measured average soil moisture levels at 10 cm depth on each sample date (n=10 samples). We found that *P. tricuspis* abundances responded to soil moisture levels in a positive manner. Seasonal abundance of P. *tricuspis* was highest from August to November and lowest in February and March. Phorid abundances in each sample plot at each study location over time indicate that P. *tricuspis* populations are synchronized at a local spatial scale, with populations in each plot following similar fluctuation patterns over time. A spatial analysis of P. *tricuspis* abundances over five weekly samples at three locations in Washington Parish, LA was done in September-October 2005. All *S. invicta* mounds in an arbitrarily defined area were located, marked, and sampled for *P. tricuspis* weekly for five consecutive weeks. Analyses of *P. tricuspis* abundances indicate: 1) abundances follow a negative binomial distribution: flies are aggregating at mound disturbances; 2) there is no spatial correlation in numbers of *P. tricuspis* appearing at fire ant mounds: phorid flies are being attracted to disturbed fire ant mounds in a random manner. It is not possible to model fly densities using a procedure such as Kriging, at least at a local spatial scale, since we did not find any evidence of spatial dependence in fly abundances.

Survey of Red Imported Fire Ant Populations in North Central Mississippi

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Introduction

The red imported fire ant (RIFA), *Solenopsis invicta* Buren occurs as two socially distinct types. RIFA colonies can either be monogyne colonies that will accept only a single queen or as polygyne colonies which contain numerous functional queens (Vinson 1997). Polygyne colonies are usually found as a collection of closely spaced mounds with RIFA workers moving freely between these mounds, whereas monogyne colonies are typically more widely spaced individual mounds.

The RIFA population in northern Mississippi has been expanding into the range of the black imported fire ant (BIFA), *Solenopsis richteri* Forel (Streett et al. 2006). Where these two species coexist, RIFA populations were found to extend further north in western Mississippi, but only as far as central Mississippi on the eastern side of the state (Figure 1).

A preliminary study was conducted to determine the distribution of the monogyne and polygyne RIFA populations in northern and central Mississippi. This information may explain the encroachment of RIFA populations into the BIFA range in northern Mississippi.

Methods

Study Site: Samples of worker ants were collected from field colonies in Mississippi. Mounds were mapped with a backpack Trimble 124 beacon DGPS system utilizing GIS Solo CE V3.0 software (TDS) installed on a Compaq iPAQ. Population estimates were made for each mound mapped in the survey using a population indexing system based on size and brood production (Harlan et al. 1981). A 1.5 ml microfuge tube containing approximately 1.0 ml of isopropanol was used to collect a sample of 25-50 ants from each mound. The samples were labeled for identification, and stored at 4 degrees C until PCR analysis.

PCR: Primers described in Valles and Porter (2003) were used to amplify Gp-9 alleles indicating monogyne or polygyne colony status. Primers were used in pairs rather than as multiplexed PCR to improve resolution.

Analysis: Field data and the results from monogyne/polygyne determinations were entered into ArcView 3.2a Geographic Information Systems (GIS) for spatial presentation.

Results

The distribution of RIFA, BIFA and hybrid imported fire ant populations in Mississippi are shown in Figure 1 (Streett et al. 2006). In this study, a total of twenty-six mounds from fifteen counties in Mississippi along the northern boundary of the RIFA populations were sampled to determine whether the colonies were monogyne or polygyne. Twelve polygyne colonies were identified along the northern boundary of FUFA populations in Mississippi (Fig. 1.) Although these polygyne mounds were detected along the entire east to west border of Mississippi, the largest concentration of polygyne mounds were found along the northwestern range of the RIFA in Mississippi. The highest number of polygyne mounds was found in Washington, Sunflower and Bolivar County located in northwestern Mississippi. However, monogyne mounds were still found north of this area.

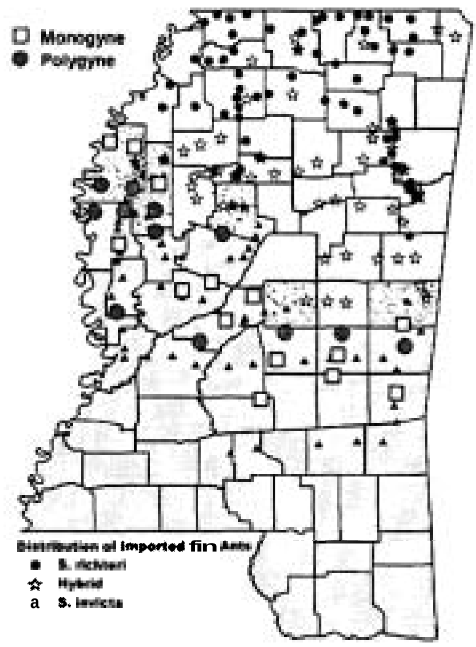

Figure 1. Survey of imported fire ant populations in Mississippi (Streett et al. 2006). Distribution of sampled monogyne and polygyne RIFA populations are superimposed.

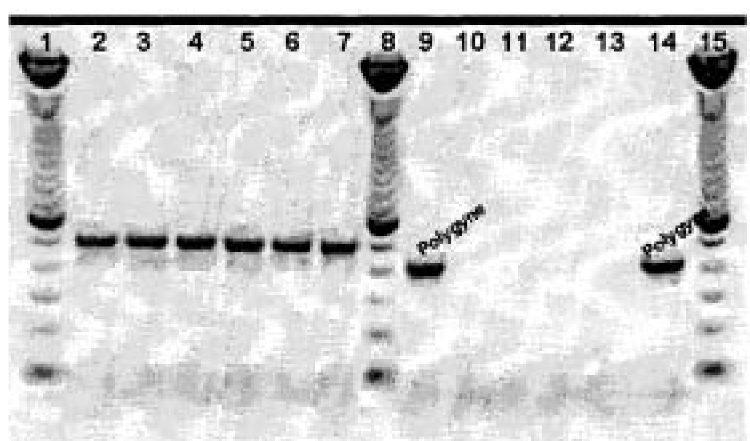

Figure 2. Representative gel with amplified DNA. Lanes: 1 ladder, 2-7 ant specimens monogyne (B) primers; 8 ladder, 9-14 ant specimens polygyne (b) primers, lane 15 ladder (all ladders are NEB100bp)

Conclusions

A majority of the polygyne RIFA colonies were found in western Mississippi. Although this is a preliminary study, the evidence suggested that the encroachment of RIFA populations further north along the western side of Mississippi may have been due to larger polygyne RIFA populations. Additional mounds will be evaluated in Mississippi to determine the distribution of monogyne and polygyne colonies in Mississippi.

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*Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

Solenopsis invicta Buren (Formicidae) and *Solenopsis richteri* Forel Mound Distribution Relationships, in Connection to the Heterogeneity of Ten Christmas Tree Farm Landscapes in Mississippi

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Abstract

Ten Christmas tree farms were selected as research sites across Mississippi. At each site a ten percent cruise was established. Plots were chosen randomly, and all imported fire ant mound positions inside the plot boundaries were captured as Global Positioning System (GPS) points. Each point, along with its corresponding mound size (cm^3) were recorded, and later uploaded into ESRI® ArcMap for analysis. Additionally, the species, height, and diameter of every Christmas tree located in the plot were recorded, along with average grass height, and other ant species collected on the farm. These data were then analyzed in SASO, to determine which variables were significantly related to mound number and mound volume. The significant variables were then used in ordinary kriging, a geo-statistical spatial interpolation method, for illustrating their influence in predicting imported fire ant mound distribution. It was found that, out of the 13 variables tested, average grass height was the only variable significantly related to mound volume and mound presence/absence. All kriging results therefore only included average grass height and average mound volume. For the majority of sites, all larger mounds were found clustered in taller grass, while the smaller mounds were scattered in larger areas of shorter grass.

The Distribution of Imported Fire Ants (Solenopsis spp.) and Their Potential Foraging Competitors in Relation to Local Conditions and Interspecific Competition in Mississippi Forests.

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Abstract (from poster) Previous studies of interactions between imported fire ants and other ant species have examined populations in open areas where they are most common. Forest habitats harbor a different group of potential competitors to imported fire ants, and interactions with these other ant species in forests are occurring under a different set of environmental conditions. We ask three questions about the distribution of imported fire ants and their potential foraging competitors in an east-central Mississippi forest: 1) Is there evidence of an influence of inter-specific competition or local conditions on their distribution? 2) How are ant communities at locations with and without imported fire ants different? 3) Which ant species are actively competing with imported fire ants for resources? To answer these questions, hybrid imported fire ants, their potential foraging competitors, and the values for a set of local conditions, were collected at locations with and without imported fire ants over a cross-section of forest habitats. Data were analyzed using a series of permutation tests that compared the observed characteristics of the ant community to those expected by random processes. The community of ant species collected at bait stations was not structured competitively, and their distribution was strongly associated with the distribution of local conditions. The assemblage of ant species was different at locations with imported fire ants from the assemblage at locations without them, but the two groups of locations were equal in richness when the number of sites sampled was standardized by rarefaction. Three species, *Monomorium* minimum, Paratrechina vividula and Pheidole bicarinata, exhibited non-random positive relationships with imported fire ant occurrence and may be competing with imported fire ants for resources. Two of those species, P. vividula and P. bicarinata, have not been considered to be competitors to imported fire ants in previous studies. A fourth species, Aphaenogaster carolinensis had a non random negative relationship with imported fire ant occurrence. An ordination of site variables with the occurrence patterns of imported fire ants and A. cavolinensis overlaid produced a clear gradient from one species habitat into the others. This indicates that the negative association between these species can be explained best by their habitat preferences and not by the negative effects of competition between them.

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Entomological, Economical and Philosophical Considerations Concerning Automated Detection and Treatment of Fire Ant *(Solenopsis* species) Colonies

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Abstract

An automated fire ant mound detection and formicidal bait application device was developed to facilitate fire ant management in areas such as pastures, some row crops (i.e. soybean), or high-profile land use areas (golf courses, state or county parks, campgrounds, etc.). Our research indicates that the number of ants attracted to pieces of hot dog in areas where individual mounds were treated with formicidal bait (Amdro) did not differ statistically from the number of ants attracted to hot dog baits in areas receiving a broadcast treatment of the insecticide. Additionally, the broadcast treatment was detrimental to the population of at least one native species (*Monomorium* minimum). This indicates that treatment of individual mounds with automated equipment is a feasible alternative to broadcast bait application from both ecological and entomological aspects.

The electronics developed to detect mounds were custom designed based on findings from previously conducted experiments using a thermal imaging system. The data produced by the thermal imaging system indicated that it was possible to detect fire ant mounds by detecting the thermal profile of the ground. It was also determined that the pixel values could be lumped to produce a much lower resolution image without loosing the efficacy of detection. The reduced pixel-count made it possible to use lower cost thermal sensors such as a 1-line array as opposed to a 2-D array for acquiring the thermal profile information, and reduced the amount of data and computing resources of the system making it more feasible to operate in real-time.

The automated system consists of an embedded controller (main controller) and one or more sensing units. The embedded controller is the central processing system that manages the operation of the sensors and other control functions and may interface with a Global Positioning System. The user interaction is done with a touch screen. The sensor unit consisted of a one-line thermal array sensor and an infrared differential pyro-electric sensor. Data from the sensor unit is sent to the main controller digitally thru an RS485 interface. The thermal array sensor produces a surface temperature profile of the ground and the differential pyro-electric sensor produces a pulse for every change in the thermal profile that is above or below ambient. The geo-referenced data is collected and stored in a flash card for off-line analysis and is used as an input to the decision making process when the system is operating in real-time mode. Processed data is then used to trigger an appropriate formicidal bait application device to apply bait to the area surrounding the detected fire ant mound.

The cost of insecticidal bait (hydromethylnon) for individual mound treatment at 19.5g per mound would cost more than broadcast applications if there were more than 47 mounds occurring per acre. Automated sensing of mounds and application of bait to individual mounds would therefore be most cost effective when mound numbers were maintained at a low level. Application costs of individual mound treatment would be restricted to bait costs only if the

application were multitasked with a mower or other agricultural unit that would be required to transverse the entire field while performing its required operation. Application by broadcast equipment would likely require additional travel over the field separate from mowing or other operation requirements. This would add the cost of fuel, equipment depreciation and operator costs to the cost of insecticide.

Evaluation of BAS 3201 Bait and Drench Applications for the Control of Imported Fire Ants

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BAS 3201 was used in three field trials conducted in the summer of 2005 in south Georgia. In the 14 day trials, BAS 3201 performed as well as Orthene when both products were applied as individual mound treatments. BAS 3201 bait applied as an individual mound treatment was superior to Advion as an individual mound treatment. BAS 3201 and Advion were similar in controlling imported fire ants when the baits were broadcast.

BAS 3201, common name Metaflumizone, is a semicarbazone that shows good potency on insect pests in several orders, including Hymenoptera. This material has been shown to be compatible with IPM and IRM practices. It has an opposite mode of action compared to pyrethoids in that it blocks the sodium channel of the nervous system causing "relaxed paralysis of insects.

Plots were established at the Fitzgerald Country Club Golf Course in early July 2005. Active colonies in each plot were marked with a wire surveyor flag. Colonies were considered active if defensive workers responded when the mound was disturbed with a wire flag. Each treatment had four replications. The application of products was made on 6 July between 9:30 and 11:30 a.m.

In the first trial, 20 ml/gal of BAS 3201 was mixed in 2 gal of water and applied to all of the active colonies in the assigned plots. Orthene was applied as a powder at 1 tbsp/md. In Trial #2, BAS 3201 and Advion baits were applied to active colonies in their respective plots, each at the rate of 1 tbsp/mound. BAS 3201 and Advion were broadcast over their respective plots at the rate of 1.5 lbs/A in Trial #3.

Post-treatment counts were made at 1, 3, 7, and 14 DAT. Post-treatment counts were made by disturbing all mounds within a plot using a wire flag. Both Orthene and BAS 3201 provided quick significant knock-down of imported fire ant colonies in the mound treatment trial. However, 3, 7, and 14 day counts were not significantly different from the untreated control for either product. In the individual mound trial with baits, BAS 3201 and Advion both provided statistically better control than the untreated plots at 1 day post-treatment. BAS 3201 maintained this significance throughout the trial. However, Advion was not significantly different from the untreated check at 3, 7, and 14 days. When applied as broadcast treatments, BAS 3201 and Advion provided significant control of imported fire ants.

In conclusion, BAS 3201 performed as well as the standard in each of the three trials conducted in July 2005. This product appears to hold promise as a candidate in the control of imported fire ants.

Evaluation of Homeowner Pormulations of Indoxacarb, Hydramethylnon, Pyriproxyfen, Fipronil, and Bifenthrin Against Red Imported Fire Ants

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Introduction

The objective of this trial was to evaluate the efficacy of broadcast applications of homeowner formulations of 0.05% pyriproxyfen bait, 0.036% hydramethylnon bait, 0.0103% granular fipronil, 0.016% indoxacarb bait, and 0.2% granular bifenthrin as compared to an untreated control. The trial consisted of 9 treatments replicated 3 times. Evaluations were conducted by collecting foraging fire ants using hot dog baited traps (Jones et. al. 1998) and by evaluating active mounds in each plot throughout the duration of the project.

Methods and Materials

Study Site: The study was conducted in Texarkana, Arkansas (Miller County) which is located in the southwest corner of the state. Density of red imported fire ant (RIFA), Solenopsis invicta, mounds at the study site was approximately 108 mounds per acre.

Design: Each treatment **and** the untreated control were replicated four times. Plots were approximately 0.25 acre in size arranged in a randomized complete block design. Plots were separated from each other by a 10 foot untreated buffer strip.

Insecticide Applications: All treatments were applied on June 7,2005. Insecticides were broadcast using a Herd spreader (Model GT-77) mounted on a Kawasaki ATV mule equipped with digital speedometer. After calibration, the specified rates were broadcast by matching to the appropriate ground speed and dispersal rate. Insecticide and application rates are listed in Table 1.

Table 1. Insecticide treatment, formulation, and application rates.

Insecticide	Formulation	Applic. Rate
UTC	-	-
Spectracide Fire Ant Bait (pyriproxyfen)	0.05% Bait	3.6 oz/1000sqft
Amdro Fire Ant Bait Yard Treatment (hydramethylnon)	0.036% Bait	0.5 lb/1000sqft
Garden Tech Over'N Out (fipronil)	0.0103% GR	2 lb/1000sqft
Spectracide Fire Ant Killer PLUS Preventer Bait Once & Done (indoxacarb)	0.016% Bait	0.5 lb/1000sqft
Ortho Max Fire Ant Killer Broadcast Granules (bifenthrin)	0.2% GR	2.3 lb/1000sqft

Fipronil treated plots and bifenthrin treated plots were "watered in" with approximately one quarter inch of water immediately following application. The test site received 0.35 inches of rain 3 days post treatment and thus all other treatments were, in effect, watered in.

Evaluation: Pre and post treatment evaluations were conducted using bait stations to collect foraging ants within each plot following the methods of Jones et al. (1998). Bait stations consisted of a 0.25 inch hot dog cube placed on a snap vial lid and marked with a wire survey flag. Ten bait stations were arranged as two transects with 5 bait stations in each transect, located approximately 4.7 m (15 feet) on each side of the center line of each plot. Bait stations within each transect were approximately 4.7 m (15 feet) apart. Bait was made available to foraging ants for approximately 30 minutes. The number of foraging RIFA collected from each station in each plot was determined to evaluate efficacy. To avoid false negative reading due to daytime heating, bait station evaluations were conducted prior to 1:00 pm.

Treatment efficacy was also evaluated by counting the number of active mounds at pre treatment and at all post treatment intervals. At the initiation of the study 10 mounds were evaluated and flagged in each plot so that the same ten mounds could be evaluated throughout the duration of the study. Mound activity was determined by gently probing mounds with a small diameter probe (minimal disturbance technique). A mound was considered active if at least 10 RIFA responded to the disturbance. This alleviated the potential for false positive (active) mounds as a result of RIFA foraging into inactive mounds. To avoid false negative reading mound activity was determined prior to 1:00 pm.

Statistical Analysis: All data were analyzed using Gylling's Agriculture Research Manager Software (ARM 7.0.3. 2003). Analysis of variance was run and Least Significant Difference ($p=0.05$) was used to separate means only when AOV Treatment P(F) was significant at the 5% level.

Results and Discussion

The mean number of foraging RIFA collected from bait stations is given in Table 2.

Table 2. Efficacy of Homeowner Insecticides in Reducing Foraging Activity of Red Imported Fire Ant In Turf. Miller Co., AR. 2005.

Treatment	Mean # of Foraging RIFA (30 min. post hotdog baiting)							
	PreTreat	2 DAT	7 DAT	14 DAT	31 DAT	62 DAT	104 DAT	155 DAT
UTC	263.3a	131.7a	201.7a	168.3a	118.3a	111.5a	56.0a	54.3a
Spectracide Fire Ant Bait 0.05% (pyriproxyfen) Bait 3.6 oz/1ooosqft	273.3a	113.3a	118.3b	98.3b	44.7bc	24.5c	18.2a	3.3a
Amdro Fire Ant Bait Yard Trt 0.036% (hydramethylnon) GR 0.5	260.0a	9.2cd	5.3c	3.3c	23.4c	44.0bc	39.3a	21.0a

lb/1000sqft								
*Garden Tech Over'N Out 0.0103% (fipronil) GR 2 lb/1000sqft	270.0a	101.7ab	169.2ab	131.0ab	60.3b	42.8bc	36.0a	0.3a
Spectracide Fire Ant Killer Plus Preventer Bait Once & Done 0.016% (indoxacarb) GR 0.5 lb/1OOOsqft	270.0a	0.0d	17.0c	9.7c	40.8bc	79.2ab	47.8a	3.8a
*Ortho Max Fire Ant Killer Broadcast Granules 0.2% (bifenthrin) GR 2.3 lb/1000sqft	253.3a	55.8bc	14.2c	12.8c	39.2bc	61.7bc	40.5a	18.4a

Means followed by same letter do not significantly differ (P=.05, LSD)
Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.
*Watered in immediately following treatment

Statistically significant reductions in the number of foraging RIFA were noted at 2DAT for hydramethylnon bait, indoxacarb bait, and bifenthrin granules. Pyriproxyfen bait and fipronil granules provided a slight numerical reduction in foraging ants at 2DAT but did not differ significantly from the untreated control. At 7 and 14 DAT, all treatments except the fipronil granular provided a significant reduction in RIFA foraging activity, with hydramethylnon bait, indoxacarb bait, and bifenthrin granules providing the greatest reduction in foraging activity. At 31 DAT, all insecticide treatments provided a significant reduction in the number of foraging RIFA compared to the untreated control with fipronil bait being significantly less effective than hydramethylnon bait. All treatments except indoxacarb bait provided a statistically significant reduction in RIFA foraging activity at 62 DAT when compared to the untreated control. No differences among treatments were observed for RIFA foraging when rated at 104 and 155 DAT.

Results of this test indicate that the homeowner can expect the quickest reduction in RIFA foraging activity following applications of indoxacarb bait, hydramethylnon bait, or bifenthrin granules. While these three treatments did not differ significantly with respect to reductions in RIFA foraging activity from 7DAT through the remainder of the test, indoxacarb bait was the only treatment to reduce foraging activity to zero and did so by 2 DAT.

The percentage of active RIFA mounds following treatment is given in Table 3.

Table 3. Efficacy of Homeowner Insecticides in Reducing the Number of Active Red Imported Fire Ant Mounds In Turf. Miller Co., AR. 2005.								
Treatment*	**% Active RIFA Mounds**							
	PreTreat	**2 DAT**	**7 DAT**	**14 DAT**	**31 DAT**	**62 DAT**	**104 DAT**	**155 DAT**
UTC	100a	97a	90a	90ab	80a	73a	53a	47ab
Spectracide Fire Ant Bait 0.05% (pyriproxyfen) Bait 3.6 oz/1OOOsqft	100a	90a	93a	97a	77a	23bc	10b	20c
Amdro Fire Ant Bait Yard Trt 0.036% (hydramethylnon) GR 0.5 lb/1000sqft	100a	80a	47b	57c	23b	17c	13b	35bc
*Garden Tech Over'N Out 0.0103% (fipronil) GR 2 lb/1000sqft	100a	100a	93a	83abc	60a	33bc	20b	21c
Spectracide Fire Ant Killer Plus Preventer Bait Once & Done 0.016% (indoxacarb) GR 0.5 lb/1000sqft	100a	47b	13c	10d	3b	3c	3b	18c
*Ortho Max Fire Ant Killer Broadcast Granules 0.2% (bifenthrin) GR 2.3 lb/1000sqft	100a	90a	77ab	63bc	73a	57ab	50a	70a

Means followed by same letter do not significantly differ (P=.05, LSD)
Mean comparisons performed only when **AOV** Treatment **P(F)** is significant at mean comparison **OSL.**
*Watered in following treatment

Indoxacarb bait was the only treatment to provide a significant reduction in active RIFA mounds at 2 DAT. The indoxacarb treatment, when compared to the untreated control, also maintained a significantly reduced number of active mounds throughout the remainder of the test. Treatment with hydramethylnon bait resulted in a significant reduction in active mounds at 14 through 104 DAT compared to the untreated control. Significant reductions in the number of active mounds did not occur with the pyriproxyfen bait and fipronil granule treatments until 62 DAT.

Based on the results of this trial, indoxacarb bait will provide the fastest reduction in numbers of foraging RIFA and the fastest reduction in the numbers of active RIFA mounds compared to other over the counter products tested. Hydramethylnon bait, as evidenced in this trial, would be almost as efficacious for RIFA control by the homeowner. Other homeowner products for controlling RIFA were less effective in providing quick control satisfaction.

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Evaluation of Hydramethylnon Bait and Various Contact Insecticide Formulations as Individual Mound Treatments Against Imported Fire Ants

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Abstract

The efficacy of individual mound treatments using hydramethylnon bait against red imported fire ants (RIFA) was compared to that of permethrin, acephate, bifenthrin, cyfluthrin, deltamethrin, lambda cyhalothrin, and tau fluvalinate. Statistically significant reductions in RIFA activity were noted one day following treatment with all of the treatments. However, reductions were greatest with acephate, bifenthrin and tau fluvalinate. By seven days post treatment, no significant differences among insecticide treatments occurred and all demonstrated significant reductions in mound activity when compared to the untreated control. This trend held through *56* days following insecticide application. With the exception of deltamethrin, all insecticide treatments had significantly reduced RIFA mound activity than the untreated control at the final evaluation (84 days following

Introduction

Arkansas homeowners frequently ask whether to use bait or contact insecticides when treating red imported fire ant (RIFA), Solenopsis invicta, mounds individually. To demonstrate the pros and cons, a study was conducted to demonstrate the speed of activity and efficacy of baits verses contact insecticides. Specifically, the speed and efficacy of 0.73% hydramethylnon bait was compared to that of permethrin, acephate, bifenthrin, cyfluthrin, deltamethrin, lambda cyhalothrin, and tau fluvalinate.

Materials and Methods

Study Site: Product evaluations were conducted in Warren, Arkansas (Bradley County) in southeast Arkansas. Density of RIFA mounds at the study site was approximately 260 mounds per acre indicating the presence of multiple queen (polygyne) colonies. Grass and other vegetation had been mowed approximately 1 week prior to initiation of the study.

Mound Selection: All visible mounds within the study area were flagged prior to initiation of the study. Flags were not placed directly into the mound but approximately two inches from the base of the mound (on the north side) to minimize disturbance. Treatment number, replicate number and mound number were permanently marked on each flag. Fire ant mounds receiving treatments were of similar size. Flags remained on all untreated mounds so that mound relocation could be determined.

Evaluation: Evaluation of fire ant populations was conducted at day 0 (day prior to treatment), day 1 (1 day post-treatment), day 2, day 3, day 7, day 14, day 28, day 56 and day 84. Fire ant activity was determined by gently probing each mound with an 1/8 inch metal probe then counting the number of ants responding within 10 seconds. The distance and activity of newly formed mounds found near treated mounds were recorded to determine percent mound relocation. The presence of fire ant brood (eggs, larva or pupa) was determined during the final evaluation period by digging into the mound.

Pesticide Application: Application rates for all treatments are listed in Table 1. Labeled methods and application rates for products currently labeled for fire ant control were used. The 1.0% cyfluthrin, 0.04% lambda cyhalothrin and 0.25% permethrin insecticide treatments were "watered in" immediately following insecticide application. Watering in was accomplished by gently sprinkling 1 gallon of water over the treated mounds. All insecticide treatments were applied August 6,2002.

Statistical Analysis: The number of red imported fire ants responding to disturbance, percentage of mounds with brood and percent mound relocation was analyzed using analysis of variance procedures based on a RCB design (Statistix 2000). The Protected LCD mean separation procedure was used to determine significant differences among means using 0.050 as the rejection level.

Results and Discussion

Significant reduction in RIFA mound activity was noted at one day post-treatment for all of the insecticide treatments (Table 2). However, the highest level of control was observed with acephate dust , bifenthrin granules and tau fluvalinate drench (97, 90, and 89% control, respectively) (Table 3). These treatments maintained a high level of control throughout the study with bifenthrin and tau fluvalinate performing better than acephate at later evaluation dates. Although significant ant reductions were seen with other treatments at day 1 post treatment the level of control was somewhat lower (43-72% control).

By seven days post treatment, no significant differences among insecticide treatments occurred and all demonstrated significant reductions in mound activity when compared to the untreated control (Table 2). This trend held through 56 days following insecticide application. With the exception of deltamethrin, all insecticide treatments had significantly reduced RIFA mound activity than the untreated control at the final evaluation (84 days following insecticide application).

Statistically significant reductions in the percentage of RIFA mounds containing brood were noted with all treatments (Table 2). Bifenthrin granules, hydramethylnon bait and lambda cyhalothrin granules demonstrated the highest reduction in the percentage of mounds containing brood with 100, 94 and 91% reduction, respectively, when compared to the untreated control (Table 3). Identical results were observed from acephate dust and the tau fluvalinate drench with an 81% reduction in mounds containing brood when compared to the control. Estimated reduction in the percentage of mounds containing brood ranged from 53 to 75% for deltamethrin, cyfluthrin, and perrnethrin.

A small number of RIFA mounds did relocate in each insecticide treatment and the untreated control. However, statistically ($a = 0.05$) significant differences among either the insecticide treatments or untreated control did not occur.

Table 1. Insecticide active ingredients, application rates and trade names.

Insecticide	Rate	Trade names
1. 0.73% hydramethylnon bait	5 tablespoons per mound	Amdro Fire Ant Bait
2.50% acephate dust	1 tablespoon per mound	Ortho Orthene Fire Ant Killer
3. *1.0% cyfluthrin dust	1 teaspoon per mound	Bayer Advanced Fire Ant Killer
4. *0.04% lambda cyhalothrin	½ cup per mound	Spectracide Fire Ant Killer granules
5. *0.25% permethrin granule	1 cup per mound	Real Kill Fire Ant Killer granules
6. 0.147% bifenthrin granule	1 cup per mound	Scotts Max Guard
7. 0.05% deltamethrin dust	1 tablespoon per mound	Enforcer Fire Ant Killer dust
8. 22.3% tau fluvalinate liquid	2/3 teaspoon per mound applied as 2 gallon drench	Mavrik Aquaflow
9. untreated control		

* denotes a treatment that was gently watered in with I gallon of water as recommended by the label.

Table 2. Mean number of red imported fire ants responding to disturbance. Brood (far right column) is the percentage of fire ant mounds containing brood (eggs larva, pupa) 84 days following treatment.

	DAY									
Insecticide	0	1	2	3	7	14	28	56	84	Brood
0.73% hydra-methylnon bait	37.9	19.8 CD	4.4CDE	1.8C	1.4B	5.0B	2.7 B	10.0 BC	5.0 CD	.03 CD
50% acephate dust	43.9	1.7D	1.3DE	0.0C	0.0B	0.6 B	0.1 B	20.0 BC	11.8BCD	.10 BCD
1.0% cyfluthrin dust	47.6	17.5CD	13.7B	19.9 B	4.4B	5.1B	4.8 B	34.4 B	30.0BC	.20 BC
0.04% lambda cyhalothrin granule	46.8	22.4BC	10.6BC	20.5B	6.4B	0.0 B	0.0 B	0.0 BC	5.6 CD	.05 CD
0.25% permethrin granule	46	18.8CD	9.0BCD	21.5B	3.2B	0.0 B	0.8 B	17.7 BC	10.0 BCD	.13 BCD
0.20% bifenthrin granule	41	6.4CD	0.3E	1.6C	0.0B	0.2 B	0.0 B	5.0 BC	0.0 D	.00 D
0.05% delta-methrin dust	53	38.2B	5.6BCDE	20.5B	1.1B	6.5B	5.6 B	35.0B	35.0AB	.25B
22.3% tau fluvalinate drench	42.5	6.7CD	4.1CDE	11.7BC	1.4B	2.6 B	0.3 B	5.0 BC	9.0 BCD	.10 BCD
Untreated control	48.1	63.3A	38.5A	55.9A	70.5A	78.8A	75.9 A	85.6A	58.8A	.53A

Table 3. Estimated percent control of red imported fire ants after individual mound treatments with selected insecticides. The brood column represents the estimated percent reduction in the proportion of mounds containing brood (eggs, larva, pupa).

	DAY								
Insecticide	1	2	3	7	14	28	56	84	Brood
0.73% hydramethylnon bait	43%	89%	97%	98%	94%	96%	88%	91%	94%
50% acephate dust	97%	97%	100%	100%	99%	100%	77%	80%	81%
1.0% cyfluthrin dust	72%	64%	64%	94%	94%	94%	60%	49%	62%
0.04% lambda cyhalothrin granule	65%	72%	63%	91%	100%	100%	100%	90%	91%
0.25% permethrin granule	70%	77%	62%	95%	100%	99%	79%	83%	75%
0.20% bifenthrin granule	90%	99%	63%	100%	100%	100%	94%	100%	100%
0.05% deltamethrin dust	40%	85%	63%	98%	92%	93%	59%	40%	53%
22.3% tau fluvalinate drench	89%	89%	79%	98%	97%	100%	94%	85%	81%

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Fire Ant Management in Poultry Houses

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Abstract:

Fire ants can be a critical factor in poultry management. Foraging in the feed bins, feeding in the houses and stinging the chickens can affect the growth and development of the young chickens through stress. The loss of feed, stinging the workers and the shorting on electrical systems and motors are a burden to the poultry farmer. The implementation of a management scheme for fire ant management on a poultry farm was requested by the poultry farmer, Mr. Billy Gains. Through the implementation of the program the farmer was able to reduce stress on his flock, reduce feed loss, and save on the reduced losses of electrical systems and motors. Other species on ants returned to the environmental system, five species were collected. A two step approach was used to manage the population. Orthene was applied to the mounds against the foundation of the poultry houses and those mounds within 10 feet of the base of the foundation. Extinguish was applied using a Herd spreader to the 17.5 acre farm and dwelling around the poultry houses. Ants were monitored pre application and monthly over a two year period. The program of fire ant management was presented to the poultry farmers at a field day. The city parish government adopted and utilized the program to manage fire ants on the public grounds maintained by the city.

Conclusion:

Fire ants can be effectively managed on a poultry farm through the use of growth regulator baits. The ant population can be reduced so that ants are no longer observed or found trailing into the feed bins or the poultry houses. Fire ants were not getting into electrical equipment and motors reducing the farmers' losses in these areas. The program has shown that fire ants can be kept below threshold throughout the entire farm and residential area by following the program of treating twice a year. Additional cattle and poultry farmers have implemented the program since its completion.

Using Population Index and Foraging Activity to Evaluate Broadcast Applications of Baits and Granular Insecticides for Imported Fire Ants

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Abstract. Two baits and two granular formulations were evaluated against imported fire ants using two sampling methods: estimating population index of individual mounds and foraging activity for entire plots. The bait Advion™ provided the most rapid control: active mounds were reduced by ca. 25% at 3 d post-treatment; PI was reduced by 88% at 7 d. Granular insecticide applications worked more slowly. The reduction in PIs in all treatments did not reach 85% or greater until 60 d. Results of hot dog sampling in small plots (10350 ft2) did not accurately estimate fire ant abundance as determined by the population index method. Hot dog bait sampling is not recommended for estimating fire ant abundance in small plots.

Introduction

Chemical insecticides for the control of imported fire ants (IFAs) are available in a variety of formulations and application techniques including granules and baits for broadcast applications (Collins and Callcott 1998, Williams et al. 2001). Disturbing mounds to determine whether or not they are active is a simple, and commonly used, method of evaluating IFA insecticide treatments. Another evaluation method uses a population index (PI) in which IFA mound activity is rated depending on the presence or absence of brood and the estimated number of workers present at the time of mound disturbance (Lofgren and Williams 1982). Using the PI technique provides more meaningful data, but it is time consuming and physically taxing. Also, because IFA brood is moved deep into the ground during warmer and cooler weather, using PI to evaluate treatments may give inaccurate results.

An alternate sampling method is needed that is simpler and convenient, yet reasonably accurate. We are particularly interested in a method that could be used by county Extension agents when conducting efficacy and demonstration trials. Quantifying worker foraging using hot dog baits compared well to the PI method in estimating IFA abundance in large field plots in the southern US (R. Pereira, USDA, pers. comm.). Similarly, Greenberg et al. (2003) found a significant correlation between IFA mound counts and foraging (estimated using sugar water consumption by foragers). During an efficacy trial of broadcasted IFA granular and bait insecticides for turfgrass, we compared the sampling method using the PI system with one that quantifies foraging by counting worker IFAs found at hot dog baits as an estimate of IFA abundance.

Materials and Methods

Treatments evaluated were the baits Advion™ (0.045% indoxacarb) and Amdro® (0.73% hydramethylnon), each applied at 1.5 lb/acre; and granular insecticides Topchoice™ (0.0143% fipronil) applied at 87 lb/acre, and Talstar® EZ (0.2% bifenthrin) applied at 100 lb/acre. Plots, located at the Lincoln Co.-Fayetteville Airport in south-central TN, were 10350 ft2 and contained at least 13 mounds of black or hybrid IFA each. Four plots were used per treatment. Treatments were broadcast with an Earthway® Ev-N-Spred Broadcast-Seeder/Spreader on May 18 (baits) and 19 (granulars), 2005.

IFA mounds'were rated using the PI method at 0, 7, 14, 30, 60 and 90 d post treatment. Reduction in PI was calculated using the equation: (Pre-treatment PI – Post-treatment PI) ÷ Pre-treatment PI. Mound activity was determined before treatment and at day 1 and 3 post treatment. IFA foraging activity was estimated using hot dog baits before treatment and at 14 and 30 d post treatment. Two-inch pieces of hot dog were placed every 24 ft in two diagonals across each plot. To reduce edge effect, no hot dogs were placed at plot comers. After 1 hour, the number of hot dog pieces with IFAs was noted.

Differences among treatments in PI and mound and foraging activity were analyzed using ANOVA for a completely randomized design. To detect a relationship between foraging and PI ratings, means of percentage of hot dog baits with ants were regressed against PI for each treatment for days 14 and 30.

Results and Discussion

By 3 d post treatment, reduction in mound activity was greatest in Advion™-treated plots (24.7%), which was significantly greater than mound activity reduction in Topchoice™-treated and control plots. Advion™ also caused the greatest reduction in PI at 7 and 14 d; Amdro® was also significantly better than the granulars at reducing IFA numbers at 14 d. Reduction in PI gradually increased for the granular treatments, and by 60 d all treatments had significantly reduced PIs by at least 85%.

Pre-treatment foraging activity was statistically similar among treatment plots. At 14 d, in contrast to the PI sampling results, hot dog sampling indicated Talstar® provided significantly better control; however, broadcasted contact insecticides are known to suppress IFA foraging activity (Jones et al. 1998). More foraging was observed in the Topchoice™ and control plots than in the Advion™ or Amdro® plots at 14 d. This was expected because the fipronil formulation used is slower-acting. With the exception of the Topchoice™-treated plots, results of hot dog sampling at 30 d did not correspond with reductions in PI for all treatments. Regression analysis detected no significant relationship between results of hot dog sampling and reductions in PI for both dates.

Size and proximity of plots may have affected results of hot dog baiting. Using small, isolated plots, Barr's (2004) IFA sampling indicated a correlation exists between mound activity and number of IFA at hot dog baits. The minimum distance between our treatment plots was –25 ft.; foraging may have occurred across plots. More importantly, the hot dog baits may have attracted IFA workers from untreated areas outside of plots. To avoid this, larger plots should be used and sampling should be conducted farther from plot edges.

Acknowledgments

The authors would like to thank Chuck Silcox and DuPont™ for partial support in conducting this study; Fayetteville-Lincoln County Regional Airport Authority for providing the test site, and Joey Morton, Michael Whittenburg, Roger Fox and Marcella Fox for assistance in conducting the study.

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Bifenthrin and Chlorpyrifos in Reduced-Radius Band Treatments for In-field Application on Nursery Plants

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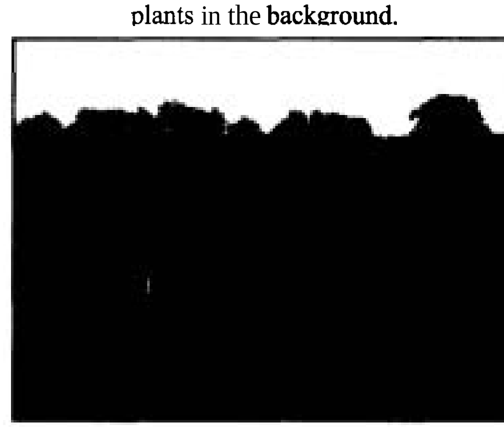
Introduction

The current treatment for pre-harvest field-grown nursery stock (in-field nursery stock), as described below, is inefficient and limited to a single insecticidal choice. Thus additional treatment methods, as well as additional approved insecticides, are needed to insure fire ant-free movement of this commodity.

USDA, APHIS is responsible for developing treatment methodologies for certification of regulated commodities for compliance with the Federal Imported Fire Ant Quarantine (7CFR 301.81). The single currently available IFA quarantine treatment for in-field nursery stock requires a broadcast application of approved bait followed in 3-5 days by a broadcast application of granular chlorpyrifos (Dursban). This treatment must extend 10 feet beyond the base of all plants to be certified. After a 30-day exposure period, plants are certified IFA-free for 12 weeks. **A** second application of granular chlorpyrifos extends the certification period for an additional 12 weeks.

Restrictions on chlorpyrifos within recent years have lead to reduced production consequently limiting its availability to growers and making compliance difficult. Furthermore, the current treatment radius frequently includes plants and soil that otherwise would not be treated because they are either not ready for harvest or are remaining within the quarantine zone. And lastly the 30-day exposure requirement is a mild inconvenience slowing commodity delivery. Thus, trials of band-applied treatments for in-field B&B were initiated to focus on examining efficacy of products other than chlorpyrifos, reduction of treated diameter, and reduction of the exposure time required prior to plant movement.

Figure 1. Field-grown nursery stock, with harvested B&B in the foreground and in-field plants in the background.



Materials and Methods

Trials examining the efficacy of bands of contact insecticide in elimination of IFA colonies have been conducted as described below from October 2002 through the end of the most recent trial in February 2006. During this time six different trials occurred using a total of nine different contact insecticides. Three plots were assigned to each treatment used, so the availability of suitably infested plots determined how many treatments were tested in each trial. Many of the tested insecticides have shown promise, but the bifenthrin and chlorpyrifos treatments have at this point participated in more of the trials and are thus the subject of this presentation.

While the end use for these treatments is in a nursery setting, trials to date have been conducted in the grassy areas adjacent to runways at low-traffic airports. These sites provide long strips of land that can accommodate several plots end-to-end and have no treatment conflicts that could occur with land used for crops or livestock. Plots were formed around 800 ft long center lines that had a minimum of five active IFA mounds within the 4 ft wide (2 ft on each side of the center line) observation strip (Figure 3). To provide a buffer zone between plots, plot center lines, which simulated rows of plant stock, were set a minimum of twenty feet apart side to side and end to end. Plots were marked with spray paint and identifying stakes for repeated observation.

Figure 3. Plot arrangement diagram

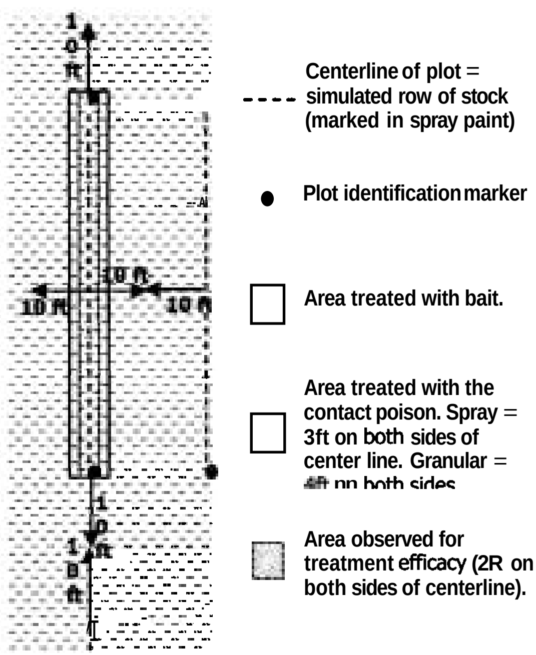
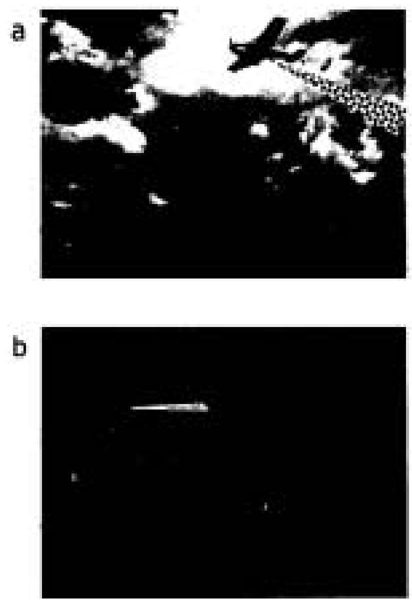


Figure 4. Application of bait (a) and contact insecticide (b) to the plots of simulated stock



Hydramethylnon bait was applied across the plots designated for chemical treatment at a 1.5 lb/A rate either through use of a shop-built spreader mounted to a farm tractor or aerially (Figure 4 a). Control plots were not treated with bait. Contact insecticide application usually occurred a week later but ranged from 28 hours to 3 weeks after baiting. Granular treatments were applied using a Gandy® 48" granular drop spreader attached to a farm tractor (Figure 4 b). Liquid treatments were applied using a roller pump boom sprayer equipped with two standard flat spray tips (8015-SS; TeeJet Corp.) to provide a 36" band spray and a total spray volume equivalent to ca. 76 gal/acre. Treatments were applied on both sides of the centerline producing a band size, depending on formulation used, either 800'x 8' or 800'x 6' in each plot. Granular and liquid formulations of bifenthrin and chlorpyrifos were applied in each of the trials from spring 2003 through spring 2005. Additionally both formulations of bifenthrin were used in the fall 2002 trial. Rates used are listed below.

Chemical	Formulation	Rate of Application
bifenthrin	granular 0.2%	200 lb/acre (0.4 lb ai/acre)
bifenthrin	flowable 7.9%	40 odacre (0.2 lb ai/acre)
chlorpyrifos	granular 2.32 or 2.5% *	260 lb/acre or 241 lb/acre (6 lb ai/acre)*
chlorpyrifos	granular 2.32%	130 lb/acre (3 lb ai/acre)†
chlorpyrifos	emulsifiable 44.8%	32 odacre (1 lb ai/acre)

* Trials from fall 2004 on use the 2.5% product since the 2.32% is no longer manufactured.
† The spring 2004 used only a half rate of the chlorpyrifos granular due to-lack of materials.

Active IFA colonies in each plot's observation area were recorded prior to bait application and again, after contact insecticide application, usually at 1, 2, 4, 6, and 8 weeks and every four weeks thereafter until reinfestation occurred or six months passed. Mounds were evaluated using as little disturbance as possible, usually through insertion of a wire flag into the mound. Mounds were considered active if any workers appeared. Rain data were collected through associated professional weather stations or simple rain gauges located at the site. Temperature was recorded during plot evaluation by air and soil thermometers.

Results and Discussion

Decline in active mounds within the observation strips was rapid for all treatments, with many of the spring trial treatments (Figures 5 & 6) having no active mounds by one week after contact insecticide application. The 2004 fall treatments still had a few lingering active mounds in the first and second weeks' observations (Figures 7 & 8). It is likely this trial had persistent mounds due to an insufficient delay (only 28 hours due to weather) between bait and contact insecticide applications, thus influencing bait uptake by IFA. Also, in the 2002 fall trial one of the bifenthrin flowable plots had a single IFA mound, mostly located under a windsock, which lingered through the trial duration. The protection provided by the cement support of the windsock in this case prevented coverage of the mound by the contact insecticide. Active mounds were also witnessed frequently in the areas between plots where only bait was applied. Decrease in control plot activity can generally be attributed to adverse weather. Spring trials started in mild weather, but the hot, arid summers in south Mississippi are known to decrease IFA activity (Callcott et al. 2000) and the trial with mound increase was a very wet summer. Likewise as the mild fall weather deepened into winter, worker activity including rebuilding mounds and response to mound disturbance would have declined with colder temperatures (Markin et al. 1974). The persistent colonies in the instances of flawed application serve to reinforce the importance of both an appropriate time frame for bait uptake and complete coverage of the treatment area with the contact insecticide.

Duration of efficacy for all treatments was strongly influenced by trial season. The spring applications of liquid chlorpyrifos indicated a minimum of 8 weeks without IFA activity in the observed areas and 10 weeks for the granular formulation (even at the halved rate used in 2004). The fall applied chlorpyrifos liquid and granular treatments, however, had no active IFA mounds for a minimum of 12 and 16 weeks respectively. Similarly, spring applications of liquid and granular bifenthrin, respectively, provided a minimum of 6 and 14 weeks without reinfestation, while fall applications of both still had no active mounds by six months after treatment.

The shorter duration of apparent IFA control in the spring trials in part may be attributed to greater reinfestation pressure than at the same observation time in fall trials. However, the fall and winter weather may also preserve actual longevity of the chemicals through inhibition of microbial degradation. While chlorpyrifos is susceptible to both abiotic and microbial factors (Baskaran et al. 1999, Miles et al. 1979), bifenthrin is considered stable after adsorption to soil except in the presence of microbial activity (USEPA 1988). Soil microbial activity is influenced by refrigeration and season (Trabue et al. 2006, Buckley and Schmidt 2003) and thus may also likewise be influenced at the field sites during winter. The fall results echo this hypothesis with the apparent effectiveness of bifenthrin treatments extending beyond the warmer weather resurgence of IFA at the end of the trial as seen in both control and chlorpyrifos treated plots.

Summary

The liquid formulation of chlorpyrifos, both formulations of bifenthrin, and a half quarantine rate of granular chlorpyrifos (only used in the 2004 spring trial) all demonstrated potential to provide quarantine level control of IFA infestation. The duration of the treatment efficacy may not have always matched or surpassed the current 10 ft radius treatment, but the reduced radius band treatments eliminated active IFA mounds from the observed treated area for six weeks to beyond six months depending on chemical, formulation, and season. All chlorpyrifos granular treated plots as well as plots treated with both bifenthrin formulations in the spring trials demonstrated 100% IFA control by two weeks, half the exposure time currently required. Liquid chlorpyrifos and the fall applied treatments may normally provide 100% control earlier than 30 days, but without observation in the third week after treatment and/or more fall trials with an appropriate delay between bait and contact insecticide application it is uncertain if an exposure shorter than four weeks would be considered in any new quarantine procedures. Future fall trials will include some of the other promising chemicals previously tested in the spring as well as incorporate chemical analysis of treated soil to support apparent periods of IFA control.

Figure 5. Efficacy of band-applied combination treatments of broadcast hydrarnethylnon bait and liquid or granular chlorpyrifos in eliminating active IFA mounds from test plots treated in the spring of 2003,2004, and 2005.

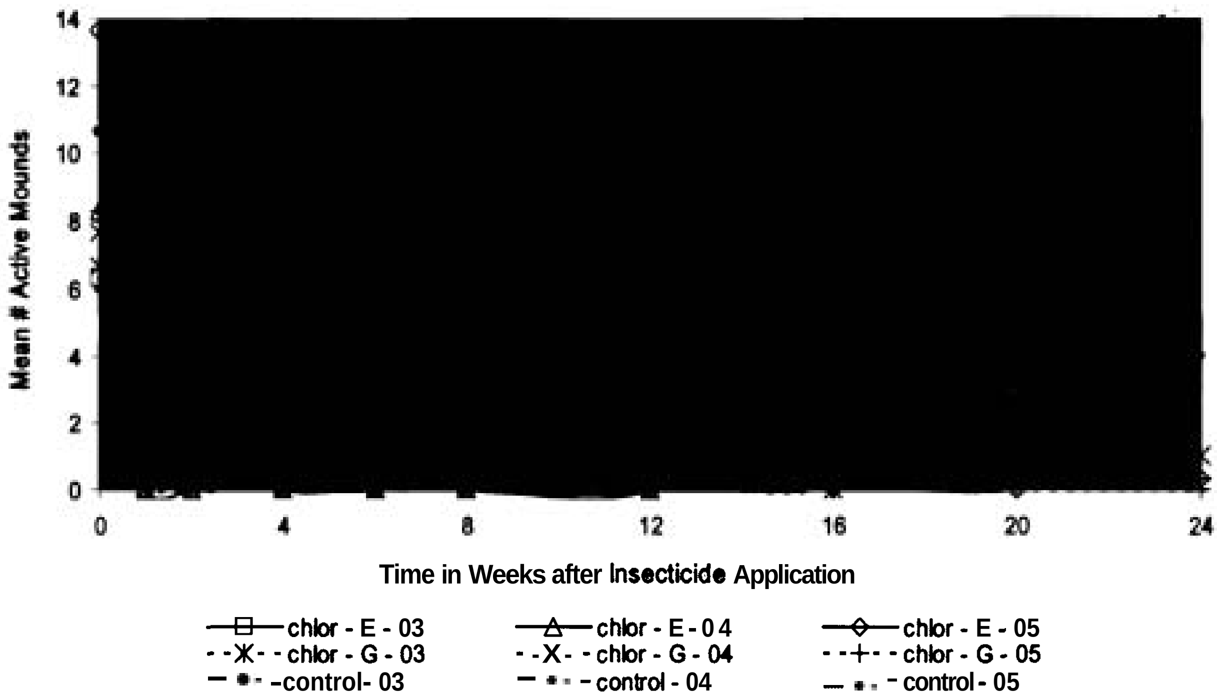


Figure 6. Efficacy of band-applied combination treatments of broadcast hydramethylnon bait and liquid or granular bifenthrin in eliminating active IFA mounds from test plots treated in the spring of 2003,2004, and 2005.

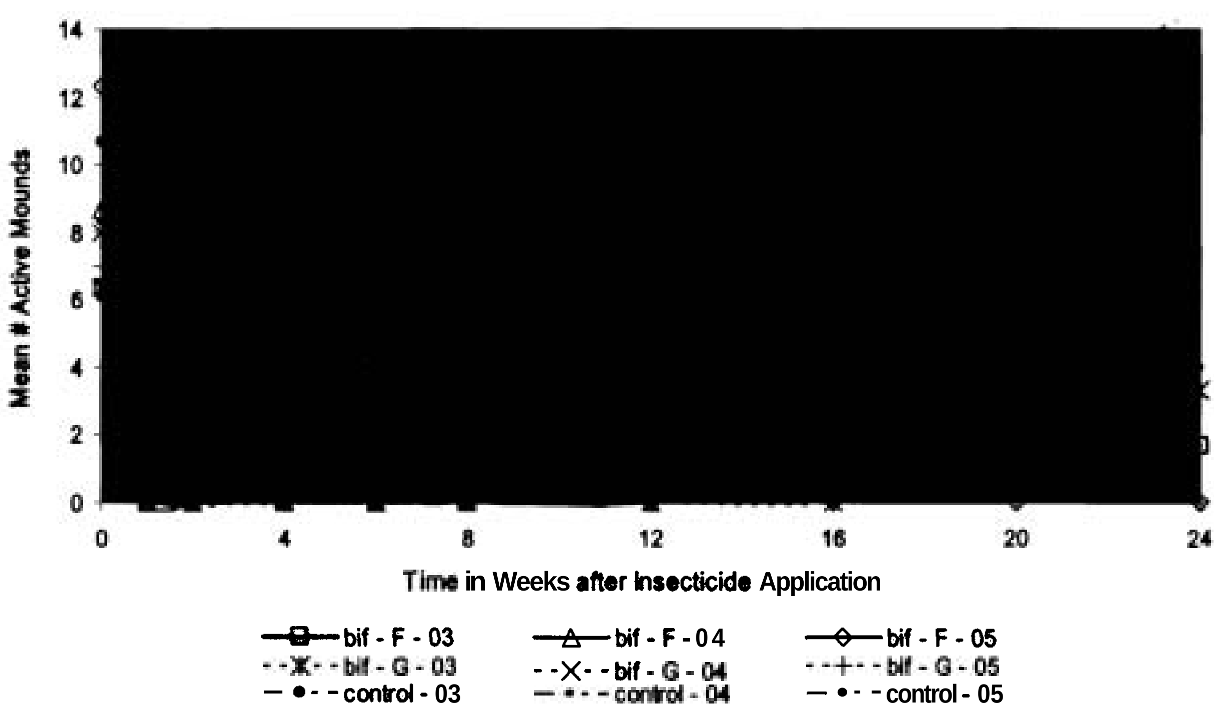


Figure 7. Efficacy of band-applied combination treatments of broadcast hydramethylnon bait and liquid or granular chlorpyrifos in eliminating active IFA mounds from test plots treated in the fall of 2003 and 2004.

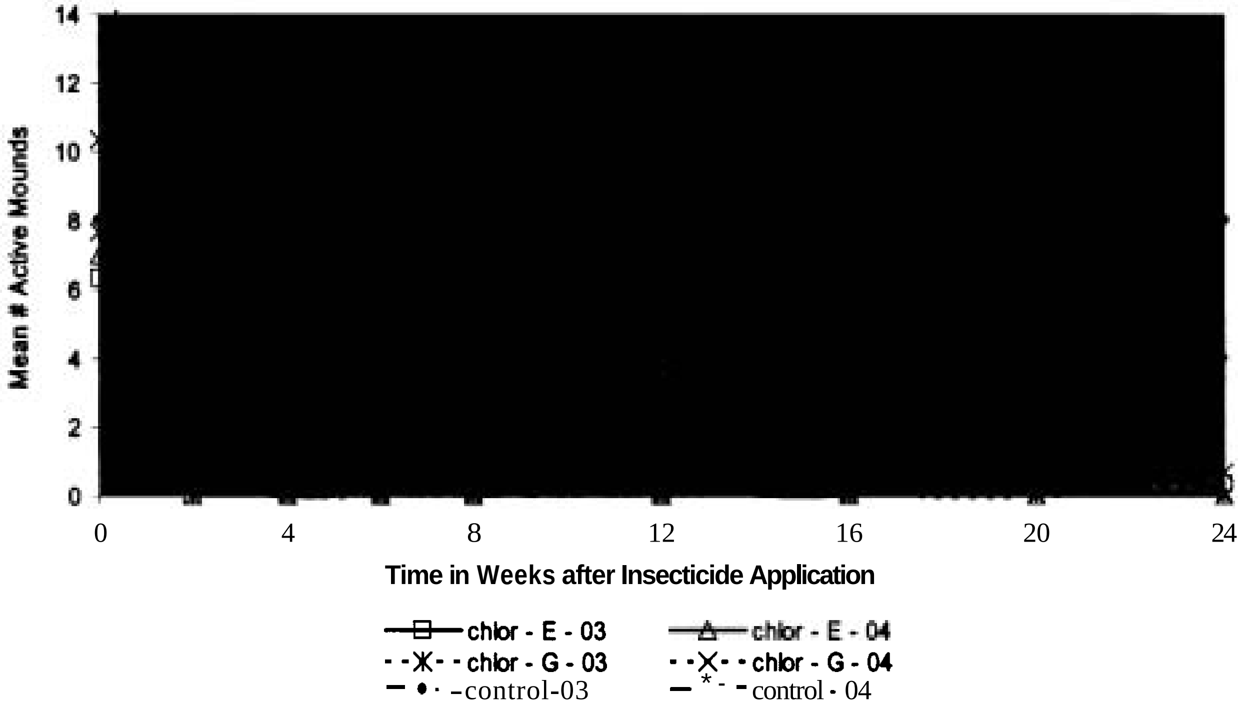
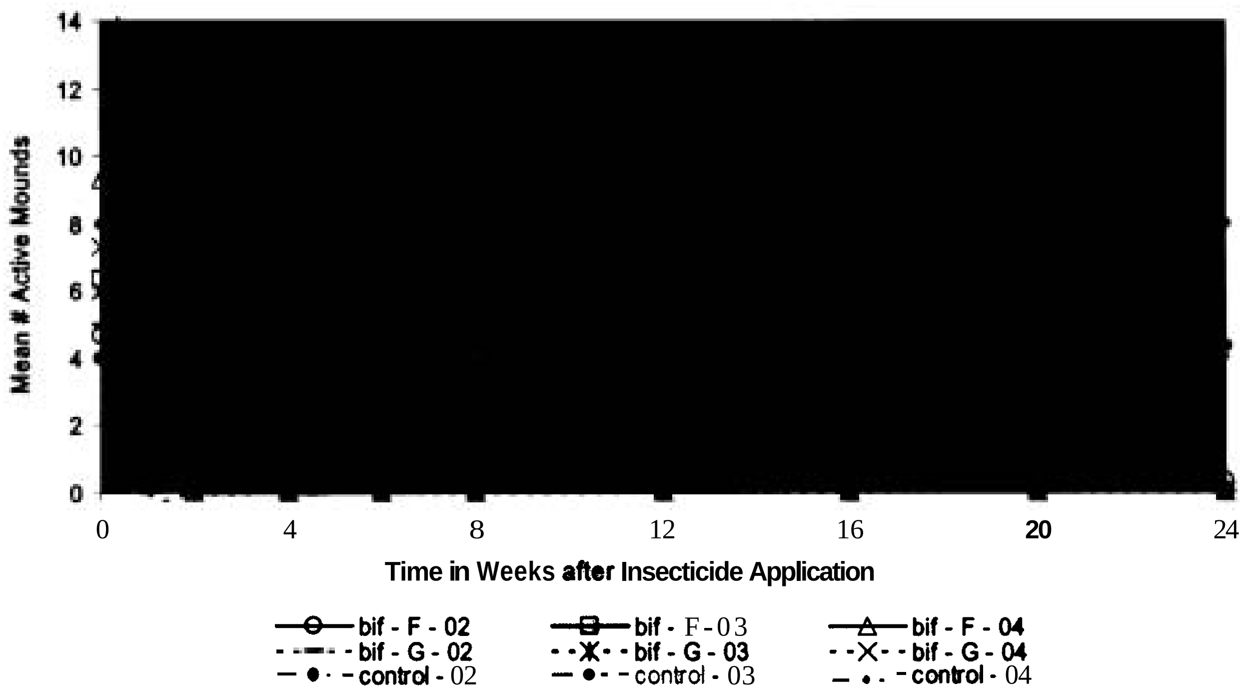


Figure 8. Efficacy of band-applied combination treatments of broadcast hydramethylnon bait and liquid or granular **bifenthrin** in eliminating active IFA mounds from test plots treated in the fall of 2002,2003, and 2004.



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Areawide Suppression of **Fire** Ants: Demonstration Project in Mississippi, 2005

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Introduction

The USDA-ARS demonstration project for the suppression of imported fire ants has entered its sixth year. In 2005, Mississippi State University joined the project monitoring sites in Clay and Grenada Counties. Two additional sites, located in Oktibbeha County, were incorporated into the Mississippi project.

The Areawide project integrates biological control agents with the chemical bait products hydromethylnon and methoprene. Mississippi's involvement in the project has focused on black/ hybrid imported fire ants (Fig. 1). The following is a report on the status of the USDA-ARS Areawide Suppression of Fire Ants Demonstration Project in Mississippi as of 2005.

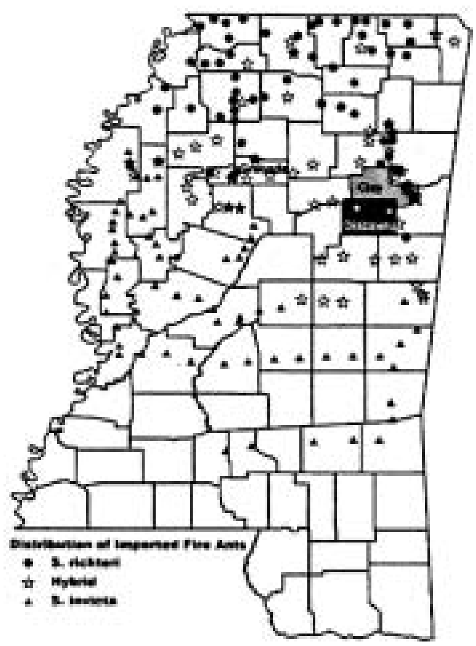

Figure 1. Distribution of imported fire ants in Mississippi. Areawide sites are located in shaded counties.

Methodology Overview

Two sites located in Grenada County were selected for chemical bait treatment and two sites located in Clay County were selected for the combined biological and chemical bait applications. Treatment areas consisted of at least 220 acres with a 280 acre minimum untreated contiguous border.

Amendments to the 2005 protocol reduced monitoring from 50 to 25 (0.1ha) plots; 10 plots in the treated areas and 15 in the boundaries. The amendment for 2005 also called for additional smaller (satellite) monitoring sites. The new sites include the MSU Golf Course and the Memorial Gardens Cemetery, Oktibbeha County (Fig. 1), with 10 and 18 (0.1ha) plots respectively. Mound counts and hotdog bait attractants were conducted every sampling period with pitfall samples collected only in the spring and fall except in satellite areas.

In cooperation with USDA-APHIS, Gulfport, Mississippi, aerial applications of Extinguish® (A.I. methoprene 0.75 lbs/acre)* and Amdro Pro® (A.I. hydromethylnon 0.75lbs/acre)* were applied when a threshold level was exceeded in the treatment areas (satellite sites are chemically treated with vehicle broadcast applications). Mound counts are no longer used as a method for determining reapplication. The current reapplication threshold is the presence of ant foragers at 35-40% of the hotdog bait attractant stations (Fig. 2).

Multiple release attempts of the biological control agents ***Thelohania solenopsae*** Knell and Allen (Microsporida) and ***Pseudacteon curvatus*** Borgmeier (Phorid fly) began in 2002 and have been monitored yearly.

Component Summary

Prima and Memorial Gardens Cemetery exceeded the reapplication threshold and were treated in October 2005.

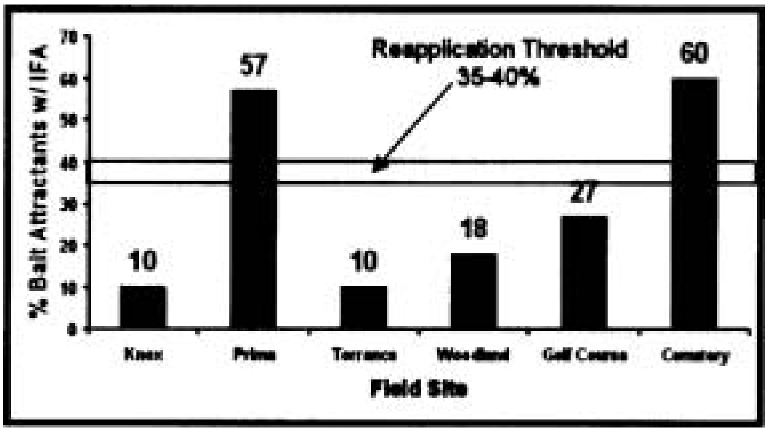

Figure 2. Percentage of hotdog bait attractants containing imported fire ants at each study site. Sites exceeding 35-40% are subject to chemical bait application.

A total of 12 ant species have been identified at the project sites (Table 1). ***Forelius pruinosus*** (Roger) was the only additional species collected in the baits at Prima in spring 2003. At this time no new formicid species have been captured. Pitfall samples from the additional sites in Oktibbeha County have not been sorted at this time.

Table 1. List of ant species captured at each of the **Areawide** study sited. Names in regular represent ants captured in bait attractants and pitfall traps. Names in Bold were only captured in pitfall traps.

Grenada County		Clay County		**Oktibbeha** County	
Torrance	Woodland	Prima	Knox	MSU Golf Course	Memorial Gardens Cemetery
Imported Fire Ant	Imported Fire Ant	Imported Fire Ant	Imported Fire Ant	Imported Fire Ant	Imported Fire Ant
Forelius pruinosus	**Forelius pruinosus**	**Forelius pruinosus**	**Monomorium** minimum	**Monomorium** minimum	**Forelius pruinosus**
Monomorium minimum	Monomorium minimum	**Monomorium** minimum	**Solenopsis molesta**	Paratrichina **vividula**	Monomorium **minimum**
Paratrichina **vividula**	Solenopsis **molesta**	**Solenopsis molesta**	**Forelius pruinosis**		Paratrichina **vividula**
Pheidole **bicarinata**	**Pheidole** dentata	Paratrichina **parvula**	Paratrichina **vividula**		
Solenopsis molesta	**Hypoponera** opacior	Paratrichlna **vividula**			
Hypoponera opacior	Paratrichina vividula	**Prenolepsis** Imparis			
Paratrichina **longicornis**					
Paratrichina **parvula**					
Pheidole **dentata**					
Tapinoma sessile					

Thelohania Summary

Since July 2002, three inoculative introductions of ***Thelohania solenopsae*** have been made at two pasture sites in Clay County, Mississippi. In 2002 and 2003, mounds were challenged with **3** grams of red imported fire ant brood inoculum and failed to yield active infected colonies. In April 2004,275 mounds were provided with **5, 8,** or 25 grams of ***Thelohania*** infected brood. In the fall of 2004, a single mound with ***Thelohania-infected*** workers was recovered at Knox. The mound was originally challenged with 8 grams of infected brood. Follow-up sampling of all active mounds located in monitored plots, in May 2005, did not yield any infected colonies. No inoculation attempts were made in 2005.

Phorid Fly Summary

The Argentina **biotype** of ***Pseudacteon curvatus*** (Fig. 4) was first released in two pastures in Clay County, Mississippi during the spring of 2002 and 2003. The phorid fly has become established on black and hybrid imported fire ants, ***Solenopsis richteri*** and ***S. invicta*** X ***richteri,*** respectively. **In** 2004 ***P. litoralis*** Borgmeier was released at Knox Farms in Clay County, Mississippi. P. ***litoralis*** was not found after 11 survey days in the fall of 2004 and four survey days in the spring of 2005. No additional releases of P. *curvatus* or ***P. litoralis*** were made in 2005.

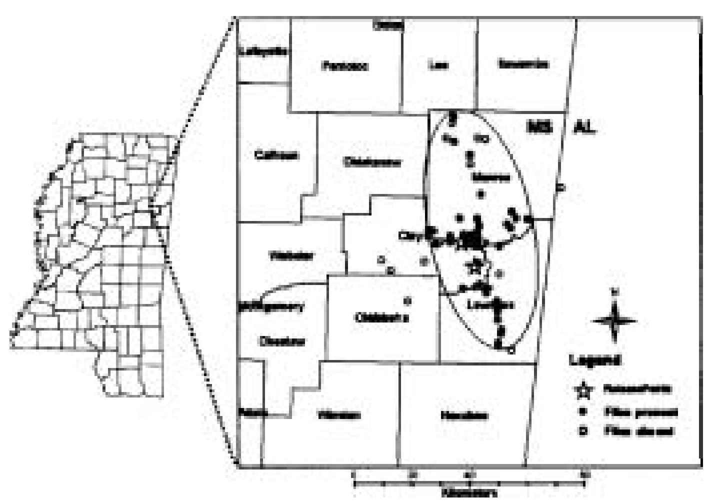

Figure 4. Establishment and spread of P. *curvatus* since release in **2002 (Thead** et al. **2005)**

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Acknowledgements

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*Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

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2006 Red Imported Fire Ant Registrants

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